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Review

Sampling methods and assays applied in *SARS-CoV-2* exposure assessment

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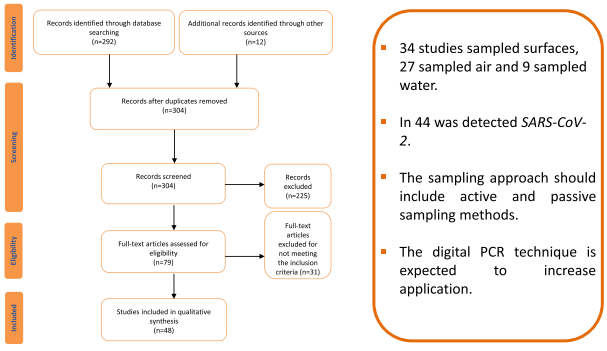
HIGHLIGHTS

- From the 48 analyzed studies, 34 sampled surfaces, 27 sampled air and 9 sampled water.
- In 44 out of the 48 studies was detected *SARS-CoV-2*.
- The sampling approach should include active and passive sampling methods.
- The digital PCR technique is expected to increase application.

GRAPHICAL ABSTRACT



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ABSTRACT

The *SARS-CoV-2* exposure assessment is critical to implement control measures and guarantee safety of patients and workers from different occupational environments. The aim of this review article was to identify methodologies applied for *SARS-CoV-2* sampling and analyses in environmental samples in different occupational and indoor environments. This study reports the search of available data published between May 29th 2020 and November 1st 2020. The search strategy used allowed the identification of 48 papers that comply with selected inclusion and exclusion criteria. The most described indoor environment consisted of health care facilities. From all the analyzed studies, 34 sampled surfaces, 27 sampled air (impactors and impingers being the most used), and 9 sampled water. All studies were based on molecular detection by qPCR of viral RNA extracted from collected samples. *SARS-CoV-2* was detected in 44 out of the 48 studies. The results suggest that the sampling approach should include both active and passive sampling methods in order to overcome each method limitations. Concerning the assays used, although most studies were based on qPCR detection, the fact that the digital PCR technique allows *SARS-CoV-2* detection at lower concentrations, indicates that this should be the chosen method for future detection studies.

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1. Introduction

A new Coronavirus - *SARS-CoV-2* - was discovered at the end of 2019, as the causal agent of severe acute respiratory syndrome. The virus has rapidly spread all over the world since then, causing a coronavirus disease 2019 (COVID-19) pandemic (Wilder-Smith et al., 2020). Scientific evidence from other microorganisms, such as *SARS* and *influenza*, report droplet and contact dissemination as the primary transmission routes (Otter et al., 2016; Wilder-Smith et al., 2020).

Hospital infections due to *SARS-CoV-2* in workers and staff has been reported, and is probably correlated to ineffective implementation of prevention and control measures (Evans et al., 2020; Gowri et al., 2004; He et al., 2020; Wilder-Smith et al., 2020). The transmission dynamics in indoor and occupational environments is likely to be multifactorial, since contaminated surfaces and air were proven to be important factors in the transmission dynamics of different viruses (Otter et al., 2013, 2016; Wilder-Smith et al., 2020). In *SARS-CoV-2*, in addition to transmission via direct contact with infected people and larger respiratory droplets, contact with contaminated surfaces or inhalation of small airborne droplets are alternative routes of infection to be considered (Morawska et al., 2020). Importantly, it has been reported that the *SARS-CoV-2* can survive on dry surfaces and in aerosols for several days to weeks (Chin et al., 2020; Van Doremalen et al., 2020).

Appropriate building engineering controls include sufficient and effective ventilation, possibly enhanced by particle filtration and air disinfection, avoiding air recirculation and preventing overcrowding. Often, such measures can be easily implemented and without much cost, as long as they are recognised as significant in contributing to infection control goals.

The *SARS-CoV-2* exposure assessment is critical to implement control measures and guarantee safety of workers from different occupational environments (Buonanno et al., 2020). Furthermore, patient safety is also a concern since several outbreaks have been reported in clinical or elderly facilities among patients and staff (Hu et al., 2020; Zhang et al., 2020). As such, it is critical to identify the best protocol regarding sampling collection and analyses. The literature indicates a wide range of sampling and analyses methods currently applied to detect *SARS-CoV-2* in the environment. The results obtained from these different studies may have implications for future policy and guidelines, highlighting the importance of a *consensus* regarding exposure assessment.

The aim of this review article was to identify methods used for *SARS-CoV-2* sampling and analyses in environmental samples in different occupational and indoor environments. This work is important to ensure both an accurate risk characterization and the development and future application of effective control measures.

2. Materials and methods

This study reports the search of available data published between September 1st 2020 and November 1st 2020. The search aimed at selecting studies on *SARS-CoV-2* in different indoor environments and included the terms "*SARS-CoV-2* in environmental samples" with English as the chosen language. The databases chosen were PubMed, Scopus, and Web of Science (WoS). This search strategy identified 292 papers in all databases. Articles that did not meet the inclusion criteria and duplicates were excluded from further analysis (Table 1).

The diagram describes the different phases of the selection of papers and the papers that were obtained in final phase (Fig. 1).

3. Results

The most described indoor environment were health care facilities (35 out of 48), followed by different environmental matrices: 9 wastewater treatment plants, rivers and household, 1 cruise, 1 household environment and 2 industrial occupational environments (Table 2).

From all the analyzed studies, 34 sampled surfaces, 27 sampled air and 9 sampled water (Table 2).

Concerning sampling methods, all surface sampling (34) was collected with swabs and all water samples (9 mentioned above) were collected in to sterile containers. Regarding air sampling it was either performed with impingers (14 out of 27), impactors (11 out of 27) or both (2 out of 27) (Ong et al., 2020; Liu et al., 2020) (Table 2).

In all the studies, molecular tool kits were used for RNA extraction which was then subject to detection by PCR methods (Table 2).

SARS-CoV-2 was detected in 44 out of the 48 articles (Table 2). Considering the environmental matrixes analyzed, *SARS-CoV-2* was detected in all the 9 articles that sampled water; in 19 of 27 articles that sampled air and in 31 of the 34 articles that sampled surfaces (Table 2).

4. Discussion

Some discussion has been raised among industrial hygienists concerning the sampling and analyses methods for *SARS-CoV-2* exposure assessment. Studies focusing on virus exposure assessment have been critically limited. This is mainly due to the difficulties in collecting and analysing airborne viruses. Among the active sampling methods, several sampling devices can be used to assess the airborne virus, being the most common the impactors and impingers, as well as filters and electrostatic precipitators (Verreault et al., 2008) (Table 2). Besides active sampling methods (air sampling), also passive methods, such as swabs, can be used. In fact, this was the sampling method mostly used in the selected papers corroborating its importance in the assessment of bioburden exposure (comprising fungi and bacteria) in health care facilities (Viegas et al., 2019) and in other indoor environments (Viegas et al., 2020).

Concerning active sampling methods, it should be stressed that longer active sampling times may be required to ensure collection of sufficient airborne viruses for detection by molecular techniques (Lednicky et al., 2020). Thus, as in other microbiologic agents' assessment, the challenge to provide a protocol from the field to bench work, will be

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 September 1st 2019	Articles published prior to September 1st 2019
Articles related to *SARS-CoV-2*	Articles not related to *SARS-CoV-2*
Articles related to environmental samples	Articles related to biological samples
Scientific original articles on the topic/journal articles	Congress abstracts, reviews, reports
Articles related to humans	Articles related to other species

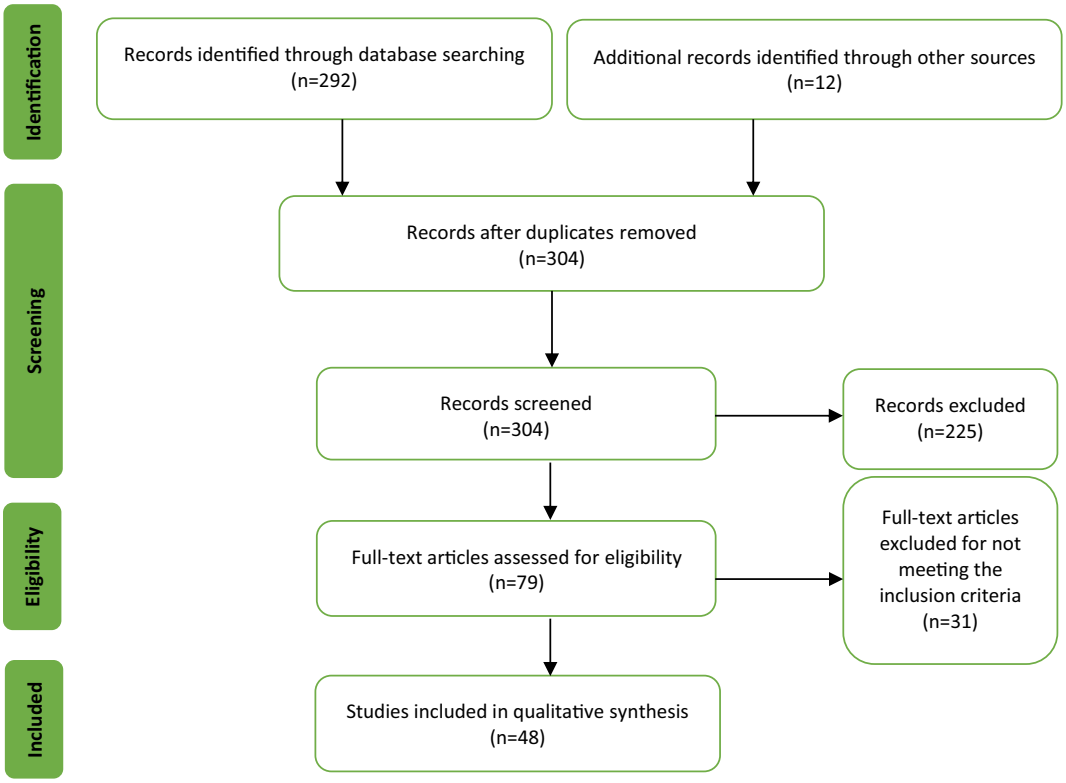

Fig. 1. PRISMA methodology of selection of papers.

to have a minimum standardized sampling volume. This is especially true for *SARS-CoV-2* detection, where there is still much to learn. As it is presented in Table 2, several methods were used for sampling with volumes ranging from 60 L (Zhang et al., 2020) to 54,720 L (Setti et al., 2020) resulting in either negative or positive detection of *SARS-CoV-2* independently of the sampling method used. From these studies the standard consensual condition is the use of an airflow rate of 200 L/min and the minimal of 1 m^3 of air during each sample collection when using Coriolis μ (impinger method device) for *SARS-CoV-2* assessment (Bertin Instruments, 2020). However, the sampling duration can affect the integrity of the virus structure and decrease their infectivity, being these drawbacks more emphasized on filters samples (Verreault et al., 2008). In fact, every virus and strains have a unique response to environmental factors, increasing the difficulty to select the optimal sampling device and conditions (Verreault et al., 2008).

Exposure assessors should acknowledge the restrictions of each active sampling method to be able to interpret their results. Due to the different performances from several devices, the results between studies can only be compared and discussed if the same sampling methods are applied, since the sampling device is of utmost importance in all bioaerosol studies (Mbareche et al., 2018). Furthermore, as it was the case of two studies (Ong et al., 2020; Liu et al., 2020), two different sampling devices to collect air samples can be used to overcome each other limitations (Viegas et al., 2019).

Impinger methods using liquid medium potentiate the viral integrity and viability and can be analyzed directly, without the need to extract the viral target solid medium or filter (Pan et al., 2019). On the contrary, the liquid used can also promote the inactivation of the *SARS-CoV-2* useful for safety reasons either in the field work as in lab work. This can be a feature also from the impinger devices, since most of the industrial hygiene laboratories facilities don't have the proper safety measures to deal with a pandemic virus, such as *SARS-CoV-2*. Furthermore, safety measures should be put in place when positive detection results were obtained, independently of the viability status of the virus.

Furthermore, the parallel use of active with passive sampling methods should be considered. In fact, air sampling is limited by short sampling periods (mostly minutes), representing only a small fraction of the bioburden exposure (Stamatelopoulou et al., 2020). Contrary, surface sampling can collect information from a larger period of time or even from several surfaces (composite samples) (Viegas et al., 2019).

Concerning the assays used to detect *SARS-CoV-2*, these were mostly based on one-step reverse transcriptase quantitative PCR detection (RT-qPCR), which is much faster than traditional PCR methods (Carter et al., 2020). The samples were extracted with different extraction kits/reagents depending on the matrix, with some of them, namely water samples, being subject to concentration prior to analysis. One to three sets of probes for different *SARS-CoV-2* viral genome regions were usually used in each assay, with positive results reporting to the amplification of all the regions subject to analysis in each particular study. The CT or cycle threshold that was considered a cut-off, above which samples were considered negative, varied within the studies, ranging from CT 38 to CT 43. A few studies (e.g. Liu et al., 2020; Gonzalez et al., 2020) have used the recently developed digital PCR technique, which has higher sensitivity and accuracy when compared to standard RT-qPCR, allowing the detection of viral nucleic acid present at low concentrations. With this method, quantification is achieved without the need of PCR cycle threshold values or standard curves. Instead, a PCR sample is portioned into droplets, with each droplet containing the target sequence being detected by fluorescent and considered positive, allowing absolute quantitation of target sequence. As the abundance of viral particles in the environment is usually low, future studies should consider this approach do detect *SARS-CoV-2* nucleic acid in environmental samples.

5. Conclusions

The most common sampling devices used to assess exposure to *SARS-CoV-2* are impactors and impingers. In addition to active sampling methods (air sampling), also swabs are being used widely in the scope of the exposure assessment. The sampling approach should include

Table 2
Data obtained from the chosen articles.

Database	Title	Country	Occupational environment study	Environment samples/samples description	Sampling methods	Analyses methods	Main findings	References
Scopus	1. Surfaces and equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the emergency department at a university hospital	France	Yes	192 Samples from hospital environment before and after decontamination.	Swabs (WHO protocol)	Viral RNA inactivation with COBAS 6800 lysis buffer and RNA Extraction and RT-qPCR with Cobas® SARS-CoV-2 Test (Roche) that targets the non-structural ORF1a/b region specific of SARS-CoV-2 and the structural protein envelope E gene.	• 10/192 total samples were positive (5.2%). • 5/45 positive samples in patient care areas (10.9%) and 4/56 positive samples on surfaces not directly in contact with patients. • After decontamination, SRARS-CoV-2 RNA remained on scuffs, stretcher, and trolleys.	(Peyrony et al., 2020)
	2. Sentinel Coronavirus Environmental Monitoring Can Contribute to Detecting Asymptomatic SARS-CoV-2 Virus Spreaders and Can Verify Effectiveness of Workplace COVID-19 Controls	Europe and USA	Yes	48 samples of high frequency touch surfaces on a New Orleans study location for preliminary tasting and 60 samples of 15 surface sites sampling on the same location	Swabs	Viral RNA extraction and Multiplex RT-qPCR essay for Coronavirus Envelope E gene and SARS-CoV-2 RdRP gene (VIR*Seek* Screen, Eurofins GeneScan)	• 4% prevalence of Coronavirus on the 48% sampled surfaces on the preliminary testing. • Detection rate of 13% on the 60 samples from the 15 sampling surfaces. • Non-symptomatic employees may be the cause of surface contamination.	(Marshall et al., 2020)
	3. SARS-Cov-2RNA found on particulate matter of Bergamo in Northern Italy: first evidence	Italy	No	Air samples from a quartz fiber filters for matriculate matter in industrial are of Bergamo (Italy)	Low gravimetric air samples (38.1 L/min for 24 h) compliant with the reference method EN12341:2014 for PM_{10} monitoring	Viral RNA extraction with quick RNA fecal soil microbe kit (Zymoresearch Ltd., 2020) qScript XLT 1-Step RT-qPCRToughMix used to detect up to three molecular marker genes (E, N, and RdRP)	• 20 out 34 RNA extractions for E, N and RdRP gene had positive result for at least one of the markers • There is evidence of SARS-CoV-2 on particulate matter.	(Setti et al., 2020)
	4. SARS-CoV-2 RNA detection of hospital isolation wards hygiene monitoring during the Coronavirus Disease 2019 outbreak in a Chinese hospital	China	Yes	Surface samples form an Isolation Intensive Care Unit, Isolation wards including cleaning area, semi-contaminated area, and contaminated area. Samples from isolation wards sewage and staff personal protective equipment	ClassiqSwabs, and collected in universal transport medium.	Viral RNA extraction and RT-qPCR SARS-CoV-2 nucleic acid detection Kit (Shanghai Berger Medical Technology Co., China)	• 3 sewage samples from the intel of pre-processing disinfection poor were positive. Negative samples in the outlet of the last disinfection pool. • No viable virus was detected by culture. • All the staff samples were negative.	(J. Wang et al., 2020)
	5. SARS-CoV-2 RNA detection in the air and on surfaces in the COVID-19 ward of a hospital in Milan, Italy	Italy	Yes	Air and surface samples from three zones classified as contaminated, semi-contaminated, and clean areas	Swabs and air samples collected using an MD8 Airport Portable Air Sampler with Gelatin Membrane Filters	Viral RNA extraction and RT-qPCR using the VET finder "Detection of CoV-19 and SARS and Recovery control in environmental sample" detection kit, which can detect both SARS-CoV-2 and SARS virus group	• Overall, 24.3% of swab samples were positive, but none of these were collected in the clean area. • The most contaminated surfaces were hand sanitizer dispensers (100.0%).	(Razzini et al., 2020)
	6. A field indoor air measurement of SARS-CoV-2 in the patient rooms of the largest hospital in Iran	Iran	No	10 air samples in hospital wards with confirmed COVID-19 patients	Impinger - containing 20 mL DMEM with 100 μg/mL streptomycin, 100 U/mL penicillin and 1% antifoam reagent for 1 h	Viral RNA extraction and collection in elution buffer, using a Vazyme Viral RNA/DNA Mini Kit (Vazyme, China) PCR amplification were performed using The SuperScript™ III One-Step RT-qPCR System with Platinum™ Taq DNA Polymerase. SARS-CoV-2 specific primer and probe sets suggested by WHO (ModularDx Kit, Wuhan Cov RdRP and E gens)	• Air samples were all negative. • Evidence of air transmission but need of more studies on sneezing and coughing emissions.	(Faridi et al., 2020)

7. SARS-CoV-2 presented in the air of an intensive care unit (ICU)	China	No	Surface and air samples from Jiangjunshan Hospital	Air sampling was performed with an WA 400 Portable viral aerosol sampler (400 L/min for 15 min). Surface sampling were collected with swabs	Viral RNA extraction with LabServ® Prefilled Viral Total NA Kit-Flex RT-qPCR assays were performed on SARS-CoV-2 open reading frame 1ab (ORF1ab) and nucleocapsid protein (NP) gene fragments using China Food and Drug Administration (CFDA)--approved commercial Nucleic Acid Diagnostic Kits (PCR-Fluorescence Probing)	• All surface samples were negative for SARS-CoV-2. • Air samples tested positive.	(Jin et al., 2020)
8. SARS-CoV-2 has been circulating in northern Italy since December 2019: Evidence from environmental monitoring	Italy	No	Sewage samples from a wastewater treatment plant	Water sampling	Viral RNA extraction was performed with Alphacoronavirus HCoV 229E (ATCC VR-740). Molecular analysis was undertaken with both nested RT-qPCR in the ORF1ab region and two published real-time RT-qPCR assays targeting the E gene of the SARS Betacoronavirus and the RdRp gene of SARS-CoV-2, respectively, as described previously (Corman et al., 2020)	• SARS-CoV-2 was detected in sewage wastewater samples.	(La Rosa et al., 2021)
9. Presence and infectivity of SARS-CoV-2 virus in wastewaters and rivers	Italy	No	18 grab samples have been collected in three WWTPs	Water sampling	Viral RNA extraction using the QIAMP Viral RNA mini kit (Qiagen). RT-qPCR performed with panel (CE-IVD, TGA and NMPA (CFDA) approved for diagnostic identification of SARS-CoV-2) containing primers and probes that target the nucleocapsid (N) gene, the ORF1ab gene and the E gene	• SARS-CoV-2 was detected in raw. • SARS-CoV-2 was not detected in treated.	(Rimoldi et al., 2020)
10. Multi-route transmission potential of SARS-CoV-2 in healthcare facilities	China	No	Samples from surface and air samples from isolation room in First Affiliated Hospitals, College of Medicine, Zhejiang University	Air sampling performed with NIOSH sampler (105-L form 30). Surface samples were collected with sterile swabs and then put onto viral transport medium	Viral RNA extraction using MagNA Pure LC 2.0 (Roche) RT-qPCR was performed using a China Food and Drug Administration--approved commercial kit specific for SARS-CoV-2 detection	• SARS-CoV-2 was detected in 1 of the 12 air samples on patient's bedside and in 4 of the 132 surface samples. • SARS-CoV-2 was also detected in 7 of 23 faeces-related air/surface/water samples. • Nosocomial infections can occur by multiple sources.	(Feng et al., 2021)
11. Hospital indoor air quality monitoring for the detection of SARS-CoV-2 (COVID-19) virus	Iran	No	Air samples from Shahid Mustafa Khomeini Hospital wards	Liquid impinger biosampler (flow rate of 12 $L \cdot min^{-1}$)	Viral RNA was extracted from the air sample impingement medium, using a GeneAll Ribospin™ and then PCR was performed with Mic Real-Time PCR System The specific primers and probes for RT-qPCR target ORF1ab and N genes (Nucleoprotein gene)	• SARS-CoV-2 was detected in 2 of the 14 air samples from different awards with positive patients. • Evidence of air transmission but need of more studies on sneezing and coughing emissions.	(Kenarkoohi et al., 2020)
12. First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan	Japan	No	Water samples from water treatment plants and river water samples.	Water collected onto 1 L plastic bottles	Viral RNA was extracted with a QIAamp Viral RNA Mini Kit (Qiagen). Used a total of six published assays, including four qPCR assays (N_Sarbeco, NIID_2019-nCOV_N, CDC-N1, and CDC-N2 assays) (Centers for Disease Control and Prevention, 2020; Corman et al., 2020; Shirato et al., 2020) and two nested PCR assays (ORF1a and S protein assays) (Shirato et al., 2020), to detect SARS-CoV-2 RNA	• SARS-CoV-2 was detected in 1 of the 5 secondary-treated wastewater samples. • SARS-CoV-2 not detected in river samples.	(Haramoto et al., 2020)
13. First detection of SARS-CoV-2 RNA in	USA	No	Water samples collected in wastewater treatment	Water samples collected in sterile 1 L Nalgene bottles	Viral RNA was extracted from the concentrated wastewater sample with	• None of the secondary treated and final effluent samples	(Sherchan et al., 2020)

(continued on next page)

Table 2 (*continued*)

Database	Title	Country	Occupational environment study	Environment samples/samples description	Sampling methods	Analyses methods	Main findings	References
	wastewater in North America: A study in Louisiana, USA			plants		a ZR Viral RNA Kit (Zymo Research) RT was performed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems), qPCR assays were performed with CDC N1 and N2 primers and probes.	tested positive for SARS-CoV-2 RNA.	
	14. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community	Australia	No	Samples from one suburban pumping station and two wastewater treatment plant in Southeast Queensland	Water samples collected with a refrigerator autosampler or a submersible in-situ high frequency autosampler and grab sampling technique	Viral RNA extraction with a combination of two kits (RNeasy PowerWater Kit and RNeasy PowerMicrobiome Kit; Qiagen) RT-qPCR used N_Sarbeco assay and NIID_2019-nCOV_N assay	• 2 samples from wastewater treatment plant were positive. • NIID_2019-nCOV_N assay failed to detect SARS-CoV-2 in any sample.	(Ahmed et al., 2020)
	15. Evaluation of the exposure risk of SARS-CoV-2 in different hospital environment	China	Yes	Air, surface samples from Xiangya hospital	Air samples collected with NIOSH (for 30 min at a flow rate of 3.5 L/minute). Surface samples collected with swabs and then inserted into a sterile EP tube containing RNAstore reagent	Viral RNA extraction carried out by resuspending using fully automatic nucleic acid extraction and purification instrument (NPA-32 P, BIOER, Hangzhou) RT-qPCR assays targeted the SARS-CoV-2 nucleocapsid protein (NP) gene fragment.	• SARS-CoV-2 detected in 7.7% of COVID-19 investigation wards and 82.6% ICUs with confirmed COVID-19 patients. • There is risk of occupational exposure.	(Ge et al., 2020)
	16. Environmental contamination of SARS-CoV-2 in healthcare premises	China	Yes	Surface samples from function zones, hospital equipment/objects and medical supplies in Zhongnan Medical Center in Wuhan	Sampling performed using Dacron swabs	RT-qPCR according to procedures recommended by the Chinese Center for Disease Control and Prevention using a SARS-CoV-2 nucleic acid detection kits to extract viral RNAs. Two different targets on the SARS-CoV-2 genome were used: the ORF1ab and N genes.	• 31.9% ICUs were contaminated. • The most contaminated objects were self-service printers (20%). • The most contaminated PPE were hand sanitizers dispensers and gloves (20.3% and 15.4%, respectively).	(Ye et al., 2020)
	17. Environmental contamination of SARS-CoV-2 during the COVID-19 outbreak in South Korea	South Korea	No	Swabs from inside and outside patient rooms in 2 hospitals A and B in Changwon	Samples collected with Dacron swabs premoistened with viral transport medium	Viral RNA extraction was performed with ExiPrep 48 Viral DNA/RNA Kit and RT-qPCR with Allplex 2019-nCoV Assay and a CFX96 Touch Real-Time PCR Detection System. This is a multiplex real-time PCR assay that detects the SARS-CoV-2 E gene, RdRp gene, and N gene.	• In hospital A: SARS-CoV-2 was detected in 17.5% samples from inside the rooms. Two samples obtained at more than 2 m from the patients showed positive results. • In hospital B: 13.6% samples from inside the rooms were positive.	(Ryu et al., 2020)
	18. Environmental contamination in the isolation rooms of COVID-19 patients with severe pneumonia requiring mechanical ventilation or high-flow oxygen therapy	South Korea	No	Air and surface samples collected from 3 negative pressure patient rooms with COVID-19 in	Samples collection with SKC BioSampler and Swab sampler	Samples were analyzed with rRT–PCR methods using PowerCheck 2019-nCoV (Kogene Biotech) which targets the SARS-CoV-2 RNA dependent RNA polymerase (RdRp) and E genes	• From 1 and 2 patient rooms, only surfaces of the endotracheal tubes tested positive for SARS-CoV-2. • In 3 patient room 13 of the 28 samples were positive. • Air samples were all negative.	(Ahn et al., 2020)
	19. Environmental contamination by SARS-CoV-2 in a designated hospital for coronavirus disease 2019	China	No	Samples collected in Wuhan 7 hospital in wards, ICU, fever clinic, clinical lab, office area and restrooms	Samples collection was performed with flocked swabs, premoistened with viral transport medium	RT-qPCR assay of SARS-CoV-2 RNA was performed using a SARS-CoV-2 nucleic acid detection kit according to the manufacturer's protocol (Shanghai ZJ Bio-Tech). Three different targets on the SARS-CoV-2 genome were amplified: the RNA-dependent RNA polymerase (RdRp), nucleocapsid (N) and the envelope (E) gene.	• 24.83% of samples were positive in medical areas. • Positive rates were 25.00% and 37.50% for the general isolation ward and intensive care unit, respectively.	(Wu et al., 2020)
	20. COVID-19 surveillance in	USA	No	Samples collected in	1 L raw wastewater was sampled	RT-ddPCR (Reverse transcription	• Detection was according to	(Gonzalez et al.,

	Southeastern Virginia using wastewater-based epidemiology			wastewater influent were aseptically collected at Hampton Roads Sanitation District in their nine major plants		droplet digital PCR) was used to enumerate SARS-CoV-2 RNA copies using three CDC diagnostic panel assays	the flowrate on population contamination.	2020)
Pubmed	21. Status of occupational protection in the COVID-19 Fangcang Shelter Hospital in Wuhan, China	China	Yes	Air and surface samples were collected in COVID-19 *Fangcang* Shelter Hospital in Wuhan, China	Air sampling was performed on four days using Air Virus collection equipment (10 min at 6 m^3/h). Surface samples were collected with swabs and placed in a virus preservation solution for transportation	PCR testing using BGI Europe A/S kit and One-step Quantitative RT–PCR system with ORF1ab target gene amplification.	• SARS-CoV-2 RNA was detected in 48 air and environmental samples after regular disinfection and cleaning.	(Zhang et al., 2020)
	22. Air, surface environmental, and personal protective equipment contamination by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) from a symptomatic patient	Singapore	No	Air and surface samples form isolation rooms	Air samples: SKC Universal Pumps (4 h at 5 L/min) and Sartorius MD8 microbiological sampler (15 min at 6 m^3/h) Surface samples: sterile premoistened swabs	RT-qPCR using SuperScriptTM III Platinum® One-step Quantitative RT-PCR system, targeted RNA-dependent RNA polymerase and E genes	• Environmental contamination detected, particularly on Toilet bowl and sink samples were positive.	(Ong et al., 2020)
	23. Bioaerosol sampling of a ventilated patient with COVID-19	USA	No	Air sample	Ten NIOSH BC 251 2-stage cyclone separated particles into 3 size fractions	Viral RNA extraction on the m2000 (Abbott Molecular), qPCR with probes for gene regions of the SARS-CoV-2 virus nucleocapsid (N1, N2, N3) and human RNase P gene.	• None of the 28 samples tested were positive for SARS-CoV-2 nucleic acid.	(Lane et al., 2020)
	24. Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals	China	No	Air samples collected in hospital	Air sampling: fixed flow rate of 5.0 L min^{-1} using a portable pump (APEX2, Casella)	Viral RNA extraction with Trizol reagent (Invitrogen). First-strand cDNA synthesis with the PrimeScript RT kit (TakaRa). Droplet-digital-PCR-based detection method (ddPCR).	• SARS-CoV-2 aerosols were mainly found to include two size ranges, one in the submicrometre region (dp between 0.25 and 1.0 μm) and the other in supermicrometre region (dp > 2.5 μm).	(Liu et al., 2020)
	25. Viable SARS-CoV-2 in the air of a hospital room 1 with COVID-19 patients	USA	No	Air samples collected from hospital room	3-hour air sampling performed using the prototype VIVAS air sampler and using a BioSpot-VIVAS BSS300P	Viral RNA extraction with QIAamp Viral RNA Mini Kit (Qiagen) RT-qPCR with primers and probe for section of the SARS-CoV-2 N-gene	• 4 air samples tested positive for SARS-CoV-2.8.	(Lednicky et al., 2020)
	26. Toilets dominate environmental detection of severe acute respiratory syndrome coronavirus in a hospital	China	No	Surface and air samples collected at the hospital	Air samples: Andersen one-stage viable impactor at 10 L/min for 30 min; 10 mL AirPort MD8 for 50 L/min for 20 min; ASE-100, 500 L/min for 2 min and WA-15 at 14 L/min for 30 min. Surface: Youkang virus sampling kit and swabs	Viral RNA extraction (NP968, Tianlong Science & Technology, Xi'an, China) and RT (Applied Biosystems QuantStudio Dx)-qPCR (Shanghai Chromysky Medical Research Co)	• 7/107 surface samples were positive. • 1/46 air samples were positive.	(Ding et al., 2020)
	27. Environmental sampling for severe acute respiratory syndrome coronavirus 2 during a COVID-19 outbreak on the diamond princess cruise ship	Japan	No	Surface and air samples from the cabins where passengers have been	Air samples: Airport MD8, Sartorius Surface: swabs	RT-qPCR MyGo Pro system (IT-IS Life Science)	• SARS-CoV-2 RNA was detected in 58 of 601 environmental samples. • SARS-CoV-2 RNA was not detected in any air samples. • SARS-CoV-2 RNA was most often detected on the floor around the toilet in bathrooms and bed pillows.	(Yamagishi et al., 2020)
	28. Air and environmental sampling for SARS-CoV-2 around hospitalized patients with coronavirus disease 2019 (COVID-19)	China	No	Surface and air samples inside airborne infection isolation rooms	Air samples: Sartorius MD8 air scan at a rate of 50 L/min (1000 L for 20 min) Surfaces: swabs	RdRp/Hel RT-qPCR with 1 μL of SARS-CoV-2 control RNA was spiked into each additional reaction.	• All air samples were negative for SARS-CoV-2.	(Cheng et al., 2020)
	29. Aerosol and surface distribution of severe acute	China	No	Surface and air samples in 2 hospital wards	Air samples: collected by using a SASS 2300 Wetted Wall Cyclone	Viral RNA extraction (LabServ® Prefilled Viral Total NA Kit-Flex and	• SARS-CoV-2 was widely distributed in air and surfaces.	(Guo et al., 2020)

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Table 2 (*continued*)

Database	Title	Country	Occupational environment study	Environment samples/samples description	Sampling methods	Analyses methods	Main findings	References
	respiratory syndrome coronavirus 2 in Hospital Wards, Wuhan, China, 2020				Sampler at 300 L/min for of 30 min. Surfaces: sterile premoistened swabs to sample	KingFisher Flex System - Thermo Fisher Scientific Inc.) RT-qPCR for SARS-CoV-2 open reading frame 1ab (ORF1ab) and nucleocapsid protein (NP) gene fragments with 2019-nCoV Nucleic Acid Diagnostic Kits (PCR-Fluorescence Probing) (Sansure Biotech Inc.) and CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.).	• SARS-CoV-2 aerosol distribution characteristics in the ICU indicate that the transmission distance of the virus might be 4 m.	
Web of Science	30. The evidence of indirect transmission of SARS-CoV-2 reported in Guangzhou, China	China	No	Aerosols and surface samples collected in multiple surfaces of 2 family houses		SARS-CoV-2 by RT-qPCR assay was performed in accordance with WHO guidelines	• SARS-CoV-2 detected in door handle. • Evidence of surface contamination and SARS-CoV-2 cross-contamination.	(Xie et al., 2020)
	31. Surface distribution of severe acute respiratory syndrome coronavirus 2 in Leishenshan Hospital in China	China	Yes	Surface samples from ICU, isolation ward in Wuhan Leishenshan Hospital	Swabs with flocked polyester tips moistened with Ringer 1/4 solution	Viral RNA extraction performed with bio robot and reaction kit from the Da'an gene qPCR was performed with PCR machine from American Roche company 480-ii;	• From 66 samples 3.03% was positive for SARS-CoV-2. • Environmental cleaning and disinfection procedures are reliable and useful on SARS-CoV-2 spreading prevention.	(Y. Wang et al., 2020)
	32. Severe acute respiratory syndrome coronavirus 2 RNA contamination of inanimate surfaces and virus viability in a health care emergency unit	Italy	No	Surface samples from emergency unit and sub-intensive care ward	Sampling performed with flexible nasopharyngeal nylon flocked swabs dipped in 3 mL universal transport medium	Viral RNA extraction performed with 200 μL of UTM™ using the QIAsymphony® instrument with QIAsymphony® DSP Virus/Pathogen Midi Kit. RT-qPCR with RNA-dependent RNA polymerase and E genes according to WHO guidelines	• From 22 samples, only 2 were positive for SARS-CoV-2 collected in external surface of continuous positive airway pressure helmets.	(Colaneri et al., 2020)
	33. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area	Spain	No	Water samples collected in WWTPs located in Region of Murcia	Sampling performed by collecting 500–1000 mL of water in sterile HDPE plastic containers	Viral RNA extracted from with Nucleo-Spin RNA virus kit (Macherey-Nagel GmbH & Co). TaqMan RT-qPCR on LightCycler 480 instrument detected three viral genes.	• 2 out of 18 secondary samples were positive for SARS-CoV-2. • This strategy is an indicator of infection within a specific population.	(Randazzo et al., 2020)
	34. SARS-CoV-2 RNA contamination on surfaces of a COVID-19 ward in a hospital of Northern Italy: what risk of transmission?	Italy	No	Surface samples from ward in University Hospital of Ferrara	Sampling performed with sterile rayon swabs pre-moistened in sterile phosphate-buffered solution	Viral RNA extraction with Patho Gene-spin Extraction kit (Generon) RT-qPCR targeted the RNA-dependent RNA polymerase (*RdRp*) gene (Generon), and the *orf1ab*, *spike* (*S*), and *nucleocapsid* (*N*) genes (ThermoFisher)	• SARS-CoV-2 was only detected in 3 samples of two floors and one-bathroom sink. • Reported to persist for a longer duration on surfaces under controlled laboratory conditions.	(D'accolti et al., 2020)
	35. SARS-CoV-2 environmental contamination associated with persistently infected COVID-19 patients	China	No	Air and surface samples collected in ICU and Isolation ward in The First Affiliated Hospital of Guangzhou Medical University	Surface samples collected with sterile flocked plastic swabs (WHO guidelines) Air sampling performed with two-stage cyclonic bioaerosol sampler developed by the NIOSH (flow rate of 3.5 L/min)	Viral RNA extraction performed with QIAGEN vRNA mini kit and SARS-CoV-2 was detected with New Coronavirus 2019-nCoV nucleic acid detection kit (Sansure Biotech Inc.).	• From 218 air samples, only on was positive for SARS-CoV-2. From 182 surface samples, 9 were positive for SARS-CoV-2.	(Lei et al., 2020)
	36. Preliminary results of SARS-CoV-2 detection in sewerage system in Niteroi municipality, Rio de Janeiro, Brazil	Brazil	No	Sewage samples collected in 12 different points in Niteroi city including WWTP	Waters samples were collected onto sterile polypropylene bottles	RNA extraction using QIAamp® Viral RNA Mini kit and QIAcube® automated system. Viral RNA detected using RT-qPCR according to CDC guidelines, detecting SARS-CoV2 – N1, N2 and N3 genes.	• SARS-CoV-2 detected in 41.6% (5/12) of raw sewage sample.	(Prado et al., 2020)

	37. Identifying the Risk of SARS-CoV-2 Infection and Environmental Monitoring in Airborne Infectious Isolation Rooms (AIIRs)	China	Yes	Air and surface samples collected from isolation rooms in Shanghai Public Health Clinic Center	Surface samples collected with sterile swabs premoistened with viral transport medium. Air samples collected with an automatic sampling system (for 1.5 h at 1 m^3/h)	Viral RNA extracted using a Magnetic beads nucleic acid isolation kit (Jiangsu Bioperfectus technologies Co.) SARS-CoV-2 was detected with RT-qPCR using the Takara One Step PrimeScript RT-qPCR kit targeting SARS-CoV-2 N gene.	• Risk of airborne transmission in isolation rooms was low (1.26%). • Viral RNA on the surface of foot-operated openers and bathroom sinks in isolation rooms.	(Song et al., 2020)
	38. Environmental contamination by SARS-CoV-2 of an imported case during incubation period	China	No	Surface samples collected prior to and after disinfection of a quarantine room	Swabs were used for surface sampling and put onto viral transport medium	Samples sent to Qingdao Municipal Center for Disease Control and Prevention for centralized RT-PCR testing for the detection of SARS-CoV-2	• 11 of the 23 of the first batch of environmental surface samples were positive for SARS-CoV-2. • 2 of 23 of the second batch of environmental samples (after first disinfection) were tested positive	(Hu et al., 2020)
	39. Detection of environmental SARS-CoV-2 RNA in a high prevalence setting in Spain	Spain	No	Surfaces and clothing samples of 10 households and 6 public service sites and in addition the wastewater from the village sewage system	Samples were collected using Dry-Sponges, pre-hydrated with 15 ml of an isotonic surfactant and virus-inactivating liquid	Viral RNA extraction using NucleoSpin RNA Virus kit (Macherey-Nagel). Detection of SARS-CoV-2 RNA by RT-qPCR targeting the envelope protein (E)-encoding gene and two targets (IP2 and IP4) of RNA-dependent RNA polymerase gene (RdRp), according to protocols included in the WHO guidelines (WHO guidelines)	• SARS-CoV-2 RNA was detected in 12% samples, including three households and three public sites.	(Fernández-de-Mera et al., 2020)
	40. Detection of Coronavirus Disease 2019 Viral Material on Environmental Surfaces of an Ophthalmology Examination Room	Turkey	No	Surface samples collected in Ophthalmology Examination Room of different surfaces around the examination chair	Dacron swabs were used to gather the surface samples.	RT-qPCR	• Two samples that were taken after examinations were found to be positive for SARS-CoV-2, 1 from the slitlamp breath shield and 1 from the phoropter.	(Aytogan et al., 2020)
	41. Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients	Singapore	No	Air and surface samples from patient rooms in isolation rooms.	Air: Six NIOSH 251 BCE bioaerosol samplers (flow-rate of 3.5 L/min for 4 h). Surface: Puritan EnviroMax Plus pre-moistened macrofoam sterile swabs	Viral RNA extraction: QIAamp viral RNA mini kit. Detection using SuperScript III Platinum One-Step RT-qPCR Kit targeting the envelope (E) genes16 and a modified orf1ab assay	• 56.7% of rooms have at least one environmental surface contaminated. • SARS-CoV-2 detected in 2 isolation rooms by air sampling.	(Chia et al., 2020)
	42. Asymptomatic COVID-19 Patients Can Contaminate Their Surroundings: an Environment Sampling Study	China	No	Air and surface samples from symptomatic and asymptomatic patients in care unit rooms.	Surface sampling: using sterile swabs. Air sampling: air sampler (FSC-1 V) (15 min at 100 L/min). Filter membrane was swabbed	RT-qPCR (Sansure Biotech) targeting open reading frame 1a or 1b (ORF1ab) and the nucleocapsid protein (N) gene.	• 44 of 112 (39.3%) surface samples were positive for SARS-CoV-2. • SARS-CoV-2 not detected in air samples. • SARS-CoV-2 detected in asymptomatic patient room.	(Wei et al., 2020)
	43. Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care	USA	No	Surface and air samples from COVID-19 patient rooms	Air sampling: Sartorius Airport MD8 air sampler operating at 50 Lpm for 15 min. Surface samples: sterile swabs	Viral RNA Extractions: using a Qiagen DSP Virus Spin Kit. RT-qPCR: using Invitrogen Superscript III Platinum One-Step Quantitative RT-qPCR System. Primers and probe used target the E gene of SARS-CoV-2.	• We detected viral contamination among all samples.	(Santarpia et al., 2020)
	44. Aerosol and environmental surface monitoring for SARS-CoV-2 RNA in a designated hospital for severe COVID-19 patients	China	No	Surface and air samples from multiple sites in Tongji Medical College, Huazhong University of Science and Technology	Aerosol samples were collected by an impingement air sampler (2400 l of air were collected at a flowrate of 80 l/min per sample). Surfaces were sampled using sterile premoistened swabs	RT-qPCR in accordance with the WHO protocol	• Only 2 swabs, sampled from the inside of a patient's mask, were positive for SARS-CoV-2. • All other swabs and aerosol samples were negative.	(Li et al., 2020)
Other sources	45. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) contamination	China	Yes	Surface and air samples from 14 temporary COVID-19 ICU in the new	15 Air samples: dry filter air sampler operating at a speed of 200	Viral RNA extracted by using the QIAamp Viral RNA Mini Kit (Qiagen). RT-qPCR targeting the RNA-dependent	• All air samples tested negative. • 2 of 128 swabs tested positive	(Cai et al., 2020)

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Table 2 (*continued*)

Database	Title	Country	Occupational environment study	Environment samples/samples description	Sampling methods	Analyses methods	Main findings	References
	in air and environment in temporary COVID-19 ICU wards			Sino-French Ward, Tongji Hospital	49 L/min for 60 min. Surfaces: 128 swabs premoistened with viral transport medium	RNA polymerase and orf1a/b gene to detect the presence of SARS-CoV-2	for SARS-CoV-2 on RT-qPCR testing.	
	46. Investigating SARS-CoV-2 surface and air contamination in an acute healthcare setting during the peak of the COVID-19 pandemic in London	UK	No	Surface and air samples collected from 7 clinical areas of the hospital	Air samples: Coriolis μ air sampler Surface: Swabs	Viral RNA extracgted by using the QIAamp Viral RNA Mini Kit (Qiagen). SARS-CoV-2 viral RNA was detected by RT-qPCR using AgPath-ID One-Step RT-PCR Reagents (Life Technologies) with specific primers and probes targeting the envelope (E)	• Viral RNA was detected on 114/218 of surfaces and 14/31 air samples but no virus was cultured.	(Zhou et al., 2020)
	47. Exhaled breath is a significant source of SARS-CoV-2 emission	China/USA	No	Air and surface samples from Hospital and Isolation room, patient personal items	Air samples (impinger methods): WA-15 (15 L/min) and WA-400 (400 L/min). Surface samples: swabs	Viral RNA Extraction with a MagMAX™ Multi-Sample 96-Well RNA Isolation Kit (Thermo Fisher Scientific). RT-qPCR targeting both ORF1ab and N genes using a detection kit (Jiangsu Bioperfectus Technologies)	• SARS-CoV-2 on 5.4% and 3.8% of surface and air samples, respectively. • COVID-19 patients can exhale the virus considering toilets and floors reservoirs.	(Ma et al., 2020)
	Clinical data on hospital environmental hygiene monitoring and medical staff protection during the coronavirus disease 2019 outbreak	China	Yes	Air and surface samples from different hospital sites	Air sampling: microbial air sampler (MAS-100 ECO) (100 L/min) Surface sampling: sterile swabs	RT-qPCR with primers and probes for two sequence regions (ORF1ab and N).	• SARS-CoV-2 detected in nurse station in the isolation area and in air samples of the same area.	(Jiang et al., 2020)

active (with an air volume of, at least 1 m^3, per sample) and passive sampling methods to overcome each method limitations.

Concerning the assays used to detect *SARS-CoV-2*, these were mostly based on one-step reverse transcriptase quantitative PCR detection (RT-qPCR), but an increase in digital PCR technique is expected, since it allows *SARS-CoV-2* detection at lower concentration ranges.

Declaration of competing interest

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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