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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

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 2019 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).

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 matrices 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
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 m³ 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 to 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 sample/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 (isyn 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, SARS-CoV-2 RNA remained on scuff, stretcher, and trolleys. | (Peyrony et al., 2020) |
| Scopus   | 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 testing 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 (VIRScreen, Eurofin) | • 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) |
| Scopus   | 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 PM10 monitoring | Viral RNA extraction with quick RNA fecal soil microbe kit (Zymoresearch Ltd., 2020) qScript XLT 1-Step RT-qPCR ToughMix 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. • 3 sewage samples from the intel of pre-processing disinfection pool 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. | (Setti et al., 2020) |
| Scopus   | 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 from 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) | • 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%). • Air samples were all negative. • Evidence of air transmission but need of more studies on sneezing and coughing emissions. | (J. Wang et al., 2020) |
| Scopus   | 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%). • Air samples were all negative. • Evidence of air transmission but need of more studies on sneezing and coughing emissions. | (Razzini et al., 2020) |
| Scopus   | 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% antimicrobial 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 (Modular Dx Kit, Wuhan CoV RdRP and E genes) | • 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%). • 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·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 wards 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, NID, 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

**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)
| Database Title | Country | Occupational environment study | Sampling methods | Environment study sample type | Vials/Specimen description | Analyses methods | Main findings |
|----------------|---------|-------------------------------|-----------------|-------------------------------|---------------------------|-----------------|--------------|
| Pena et al., 2021 | South Korea | South Korea | Air and surface samples collected from negative pressure patient rooms in hospital A and B in Changwon | Samples collected with Dacron swabs | 2 m from the patients showed positive results. | RT-PCR, multiplex real-time PCR assay that detects the SARS-CoV-2 E, ORF1ab, and N genes. | Viral RNA extraction was performed using a High Capacity cDNA Reverse Transcription Kit. qPCR assays were performed with CDC N1 and N2 primers and probes. | From 1 and 2, patients from the same ward with COVID-19 in the community were detected positive for SARS-CoV-2. No samples from wastewater in North America were tested positive for SARS-CoV-2. South Korea was the only country positive for SARS-CoV-2. |
| Study                                                                 | Country | Region | Sample Type                        | Sampling Method                                                                 | Virus Detection Method                                       | Findings                                                                                       |
|---------------------------------------------------------------------|---------|--------|------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Southeastern Virginia using wastewater-based epidemiology            |         |        | Wastewater samples                 | SARS-CoV-2 RNA was detected in 48 air and environmental samples after regular disinfection and cleaning. | RT-qPCR using SuperScriptTM III Platinum® One-step Quantitative RT-PCR system. | (Zhang et al., 2020)                                                                         |
| 21. Status of occupational protection in the COVID-19                | China   | Yes    | Air and surface samples            | Air sampling was performed on four days using Air Virus collection equipment (10 min at 6 m³/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.                                    |
| Fangcang Shelter Hospital in Wuhan, China                           |         |        |                                    |                                                                                 |                                                               | (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 M88 microsampling sampler (15 min at 6 m³/h). Surface samples: sterile premoistened swabs | Viral RNA extraction with 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. | - 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 | 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 Viral RNA extraction (NPS68, Tianlong Science & Technology, Xian, China) and RT (Applied Biosystems QuantStudio Dx)-qPCR (Shanghai Chromysky Medical Research Co) | - 4 air samples tested positive for SARS-CoV-2.8. - 7/107 surface samples were positive. - 1/46 air samples were positive. | (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 with QIAamp Viral RNA Mini Kit (Qiagen). RT-qPCR with primers and probe for section of the SARS-CoV-2 N-gene Viral RNA extraction (NPS68, Tianlong Science & Technology, Xian, 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-LS 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/He1 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 (LabSero® Prefilled Viral Total NA Kit-Flex and | SARS-CoV-2 was widely distributed in air and surfaces. | (Guo et al., 2020) |
SARS-CoV-2 aerosol distribution characteristics in the ICU indicate that the transmission distance of the virus might be 4 m.

**Table 2 (continued)**

| Database | Title | Country | Occupational environment study | Environment sample/samples description | Sampling methods | Analyses methods | Main findings | References |
|----------|-------|---------|---------------------------------|---------------------------------------|-----------------|-----------------|--------------|------------|
| P. Pena, J. Morais, A. Quintal Gomes et al. Science of the Total Environment 775 (2021) 145903 | 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 | 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 by RT-qPCR assay was performed in accordance with WHO guidelines | • SARS-CoV-2 aerosol distribution characteristics in the ICU indicate that the transmission distance of the virus might be 4 m. | (Xie et al., 2020) |
| P. Pena, J. Morais, A. Quintal Gomes et al. Science of the Total Environment 775 (2021) 145903 | 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 RT-qPCR machine from American Roche company 480-ii; | • SARS-CoV-2 detected in door handle. • Evidence of surface contamination and SARS-CoV-2 cross-contamination. 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. From 22 samples, only 2 were positive for SARS-CoV-2 collected in external surface of continuous positive airway pressure helmets. | (Y. Wang et al., 2020) |
| P. Pena, J. Morais, A. Quintal Gomes et al. Science of the Total Environment 775 (2021) 145903 | 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 QIAamp® DSP Virus/Pathogen Midi Kit. RT-qPCR with RNA-dependent RNA polymerase and E genes according to WHO guidelines Viral RNA extraction performed from with Nucleo-Spin RNA virus kit (Macherey-Nagel GmbH & Co), TaqMan RT-qPCR on LightCycler 480 instrument detected three viral genes. Viral RNA extraction with PathoGene-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) | • 2 out of 18 secondary samples were positive for SARS-CoV-2. • This strategy is an indicator of infection within a specific population. • 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. From 218 air samples, only 2 were positive for SARS-CoV-2 collected in external surface of continuous positive airway pressure helmets. | (Colaneri et al., 2020) |
| P. Pena, J. Morais, A. Quintal Gomes et al. Science of the Total Environment 775 (2021) 145903 | 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 extraction performed from with nucleo-spin RNA virus kit (Macherey-Nagel GmbH & Co), TaqMan RT-qPCR on LightCycler 480 instrument detected three viral genes. Viral RNA extraction with PathoGene-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). | • 2 out of 18 secondary samples were positive for SARS-CoV-2. • This strategy is an indicator of infection within a specific population. • 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. From 218 air samples, only 2 were positive for SARS-CoV-2 collected in external surface of continuous positive airway pressure helmets. | (Randazzo et al., 2020) |
| P. Pena, J. Morais, A. Quintal Gomes et al. Science of the Total Environment 775 (2021) 145903 | 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 performed from with Nucleo-Spin RNA virus kit (Macherey-Nagel GmbH & Co), TaqMan RT-qPCR on LightCycler 480 instrument detected three viral genes. Viral RNA extraction with PathoGene-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). | • 2 out of 18 secondary samples were positive for SARS-CoV-2. • This strategy is an indicator of infection within a specific population. • 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. From 218 air samples, only 2 were positive for SARS-CoV-2 collected in external surface of continuous positive airway pressure helmets. | (D’accolti et al., 2020) |
| P. Pena, J. Morais, A. Quintal Gomes et al. Science of the Total Environment 775 (2021) 145903 | 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) Waters samples were collected onto sterile polypropylene bottles | Viral RNA extraction performed with QIAamp® Viral RNA Mini kit and QIAcube® automated system. Viral RNA detected using RT-qPCR according to CDC guidelines, detecting SARS-CoV-2 – N1, N2 and N3 genes. | • SARS-CoV-2 detected in 41.6% (5/12) of raw sewage sample. | (Lei et al., 2020) |
| P. Pena, J. Morais, A. Quintal Gomes et al. Science of the Total Environment 775 (2021) 145903 | 36. Preliminary results of SARS-CoV-2 detection in sewage system in Niterói municipality, Rio de Janeiro, Brazil | Brazil | No | Sewage samples collected in 12 different points in Niterói city including WWTP | RNA extraction using QIAamp® Viral RNA Mini kit and QIAcube® automated system. Viral RNA detected using RT-qPCR according to CDC guidelines, detecting SARS-CoV-2 – 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 (AIRs)

**China** Yes  
Air and surface samples collected from isolation rooms in Shanghai Public Health Clinic Center  
Viral RNA extracted using a Magnetic bead 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. Samples sent to Qingdao Municipal Center for Disease Control and Prevention for centralized RT-PCR testing for the detection of SARS-CoV-2.  
- **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.**  
- **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** (Song et al., 2020)

### 38. Environmental contamination by SARS-CoV-2 of an imported care 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  
- **SARS-CoV-2 RNA was detected in 12% samples, including three households and three public sites.** (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 Dacron swabs, pre-hydrated with 15 ml of an isotonic surfactant and virus-inactivating liquid  
Viral RNA extraction using NuclinoSpin 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)  
- **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.**  
- **56.7% of rooms have at least one environmental surface contaminated.**  
- **SARS-CoV-2 detected in 2 isolation rooms by air sampling.**  
- **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.**  
- **We detected viral contamination among all samples.** (Aytogan 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  
- **SARS-CoV-2 RNA was detected in 12% samples, including three households and three public sites.**  
- **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.**  
- **56.7% of rooms have at least one environmental surface contaminated.**  
- **SARS-CoV-2 detected in 2 isolation rooms by air sampling.**  
- **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.**  
- **We detected viral contamination among all samples.** (Chi a 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 samples (flow-rate of 3.5 L/min for 4 h). Surface: Puritan EnviroMax Plus pre-moistened macrofoam sterile swabs  
Viral RNA extraction using Qiagen Viral RNA Mini kit. Detection using SuperScript III Platinum One-Step RT-qPCR Kit targeting the envelope (E) genes16 and a modified orf1ab assay  
RT-qPCR (Sansure Biotech) targeting open reading frame 1a or 1b (ORF1ab) and the nucleocapsid protein (N) gene. (Santarpia et al., 2020)  
- **SARS-CoV-2 RNA was detected in 12% samples, including three households and three public sites.**  
- **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.**  
- **56.7% of rooms have at least one environmental surface contaminated.**  
- **SARS-CoV-2 detected in 2 isolation rooms by air sampling.**  
- **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.**  
- **We detected viral contamination among all samples.** (Fernández-de-Mera 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.  
Air: Six NIOSH 251 BCE bioaerosol samples (flow-rate of 3.5 L/min for 4 h). Surface: Puritan EnviroMax Plus pre-moistened macrofoam sterile swabs  
Viral RNA extraction using Qiagen Viral RNA Mini kit. Detection using SuperScript III Platinum One-Step RT-qPCR Kit targeting the envelope (E) genes16 and a modified orf1ab assay  
RT-qPCR (Sansure Biotech) targeting open reading frame 1a or 1b (ORF1ab) and the nucleocapsid protein (N) gene. (Santarpia et al., 2020)  
- **SARS-CoV-2 RNA was detected in 12% samples, including three households and three public sites.**  
- **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.**  
- **56.7% of rooms have at least one environmental surface contaminated.**  
- **SARS-CoV-2 detected in 2 isolation rooms by air sampling.**  
- **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.**  
- **We detected viral contamination among all samples.** (Fernández-de-Mera 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: Santorius Airport MDB 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. 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)

### 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 impinging air sampler (2400 l of air were collected at a flow rate of 80 L/min per sample). Surface samples were sampled using sterile premoistened swabs  
- **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)

### 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 Other sources

- **Viral RNA extracted by using the QiAamp Viral RNA Mini Kit (Qagen). RT-qPCR targeting the RNA-dependent** (Cai et al., 2020)

(continued on next page)
Ma et al., 2020

RT-qPCR with primers and probes for
SARS-CoV-2 on 5.4% and 3.8%
two sequence regions (ORF1ab and N).

COVID-19 patients can exhale
of surface and air samples,
respectively. Therefore, surface samples and air samples, respectively,
can be used to study SARS-CoV-2 transmission.

Viral RNA was detected on
the virus considering toiletsand
floors reservoirs.

Clinical data on hospital
Viral RNA Extraction with a MagMAX™
Multi-Sample 96-Well RNA IsolationKit (Thermo Fisher Scientific).

RT-qPCR targeting both ORF1ab and Ngenes using a detection kit (JiangsuBioperfectus Technologies).

Air samples: Coriolis μ air
WA-15 (15 L/min) and WA-400(400 L/min).Surface samples: swabs
collected from 7 clinicalareas of the hospital

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Viral RNA extracted by using the
WA-15 (15 L/min) and WA-400 (400 L/min) air-samplers.

Surface sampling: sterile swabs

Table 2 continued

| Database Title |
|----------------|
| China, USA | No |
| UK | No |
| China | Yes |

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significant source of
SARS-CoV-2 emission
in London during the peak of the pandemic.

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Declarations of competing interest

The authors certify that they have NO affiliations with or involve-
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affiliations, knowledge or beliefs) in the subject matter or materials
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