SYSTEMATIC REVIEW

SARS-CoV-2 and the role of airborne transmission: a systematic review [version 1; peer review: 1 not approved]

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Abstract

Background: Airborne transmission is the spread of an infectious agent caused by the dissemination of droplet nuclei (aerosols) that remain infectious when suspended in the air. We carried out a systematic review to identify, appraise and summarise the evidence from studies of the role of airborne transmission of SARS-CoV-2.
Methods: We searched LitCovid, MedRxiv, Google Scholar and the WHO Covid-19 database from 1 February to 20 December 2020 and included studies on airborne transmission. Data were dual extracted and we assessed quality using a modified QUADAS 2 risk of bias tool.
Results: We included 67 primary studies and 22 reviews on airborne SARS-CoV-2. Of the 67 primary studies, 53 (79%) reported data on RT-PCR air samples, 12 report cycle threshold values and 18 copies per sample volume. All primary studies were observational and of low quality. The research often lacked standard methods, standard sampling sizes and reporting items. We found 36 descriptions of different air samplers deployed. Of the 42 studies conducted in-hospital that reported binary RT-PCR tests, 24 (57%) reported positive results for SARs-CoV-2 (142 positives out of 1,403 samples: average 10.1%, range 0% to 100%). There was no pattern between the type of hospital setting (ICU versus non-ICU) and RT-PCR positivity. Seventeen studies reported potential air transmission in the outdoors or in the community. Seven performed RT-PCR sampling, of which two studies report weak positive RNA samples for 2 or more genes (5 of 125 samples positive: average 4.0%). Ten studies attempted viral culture with no serial passage for viral culture.
Conclusion: SARS-CoV-2 RNA is detected intermittently in the air in various settings. Standardized guidelines for conducting and reporting research on airborne transmission are needed. The lack of recoverable viral culture samples of SARS-CoV-2 prevents firm conclusions over airborne transmission.

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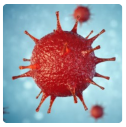
Keywords
SARs-CoV-2, transmission, COVID, Airborne



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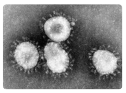

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Competing interests: CH holds grant funding from the NIHR School of Primary Care Research, the NIHR BRC Oxford and the World Health Organization for a series of Living rapid review on the modes of transmission of SARs-CoV-2 reference WHO registration No2020/1077093. He has received expenses and fees for his media work. He receives expenses for teaching EBM and is also paid for his GP work in NHS out of hours (contract Oxford Health NHS Foundation Trust) and for appraising treatment recommendations in non-NHS settings. He is the Director of CEBM and is an NIHR Senior Investigator. TJ was in receipt of a Cochrane Methods Innovations Fund grant to develop guidance on the use of regulatory data in Cochrane reviews (2015-018). In 2014–2016, he was a member of three advisory boards for Boehringer Ingelheim. TJ was a member of an independent data monitoring committee for a Sanofi Pasteur clinical trial on an influenza vaccine. TJ is occasionally interviewed by market research companies about phase I or II pharmaceutical products for which he receives fees (current). TJ was a member of three advisory boards for Boehringer Ingelheim (2014-16). TJ was a member of an independent data monitoring committee for a Sanofi Pasteur clinical trial on an influenza vaccine (2015-2017). TJ is a relator in a False Claims Act lawsuit on behalf of the United States that involves sales of Tamiflu for pandemic stockpiling. If resolved in the United States' favour, he would be entitled to a percentage of the recovery. TJ is co-holder of a Laura and John Arnold Foundation grant for the development of a RIAT support centre (2017-2020) and Jean Monnet Network Grant, 2017-2020 for The Jean Monnet Health Law and Policy Network. TJ is an unpaid collaborator to the project Beyond Transparency in Pharmaceutical Research and Regulation led by Dalhousie University and funded by the Canadian Institutes of Health Research (2018-2022). TJ consulted for Illumina LLC on next-generation gene sequencing (2019-2020). TJ was the consultant scientific coordinator for the HTA Medical Technology programme of the Agenzia per i Serivizi Sanitari Nazionali (AGENAS) of the Italian MoH (2007-2019). TJ is Director of Medical Affairs for BC Solutions, a market access company for medical devices in Europe. TJ is funded by NIHR UK and the World Health Organization (WHO) to update Cochrane review A122, "Physical Interventions to interrupt the spread of respiratory viruses". TJ is funded by Oxford University to carry out a living review on the transmission epidemiology of COVID-19. Since 2020, TJ receives fees for articles published by The Spectator and other media outlets. TJ is part of a review group carrying out "Living rapid literature review on the modes of transmission of SARS-CoV-2 (WHO Registration 2020/1077093-0)". He is a member of the WHO COVID-19 Infection Prevention and Control Research Working Group. DE has been awarded U.S. patents as a co-inventor of related oncolytic virus technologies and is a co-owner of Prophysis Inc., which retains a partial interest in the licensing rights for these technologies. JMC holds grants from the Canadian Institutes for Health Research on acute and primary care preparedness for COVID-19 in Alberta, Canada and was the primary local Investigator for a Staphylococcus aureus vaccine study funded by Pfizer for which all funding was provided only to the University of Calgary. He also received support from the Centers for Disease Control and Prevention (CDC) to attend an Infection Control Think Tank Meeting. AP holds grant funding from the NIHR School of Primary Care Research. IJO, EAS, and JB have no interests to disclose.

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Introduction

Airborne transmission is defined as the spread of an infectious agent caused by the dissemination of droplet nuclei (aerosols) that remain infectious when suspended in air over long distances and time[1]. A collection of particles (liquid or solid) ranging in size from 0.001 μm to over 100 mm suspended in a gas defines an aerosol[2]. Droplet nuclei are airborne residue (with or without embedded pathogens) of a respiratory droplet containing non-volatile solutes, from which water has evaporated to the point of equilibrium with the ambient relative humidity defines[3].

Airborne transmission via droplets and aerosols enables some viruses to spread efficiently among humans, causing outbreaks that are difficult to control. Many studies, however, often report inconclusive findings as many outbreaks are studied retrospectively and evidence to inform transmission from controlled experiments is often not available[4,5]. Among case clusters for which airborne transmission is hypothesised, published detailed investigations cannot rule out that droplet and fomite transmission could also explain human-to-human transmission[6]. Therefore, we aimed to systematically review the airborne transmission evidence for SARS-CoV-2.

Methods

We are undertaking a series of living systematic reviews investigating factors and circumstances that impact the transmission of SARS-CoV-2, based on our published protocol last updated on 1 December 2020 (archived protocol: *Extended data:* Appendix 1[7]) Briefly, this review aims to identify, appraise, and summarize the evidence (from studies peer-reviewed or awaiting peer review) relating to the role of airborne transmission of SARS-CoV-2 and the factors influencing transmissibility.

We searched four main databases: LitCovid, medRxiv, Google Scholar and the WHO Covid-19 database for COVID-19 using the terms Airborne: aerosol OR airborne OR airbourne OR inhalation OR air OR droplet) from 1 February 2020 up to 20 December 2020 (see *Extended data:* Appendix 2 for the search strategies[7]). Searches were updated every two weeks. We aimed to include sampling for the detection of SARs-CoV-2 in the population or the environment on airborne transmission. Studies can be observational including case series, ecological, or prospective; or interventional including randomised trials and clinical reports, outbreak reports, case-control studies, experimental studies, non-predictive modelling. Studies should include sampling for the detection of SARs-CoV-2. Studies on factors influencing transmission are included, such as location settings, meteorological or immunological factors. Studies incorporating models to describe observed data were eligible. Studies reporting solely predictive modelling were excluded. For relevant articles citation tracking was undertaken. We searched the included studies of all retrieved reviews and included them in the results section for reference.

We included field studies that included airborne sampling for SARs-CoV-2 in the population or the environment. JB performed the searches, TJ and ES performed the first screen and CH checked these initial screening of studies. One reviewer (ES) extracted data for each study, and a second reviewer (CH) checked and edited the extraction. We extracted information on the study characteristics, the study population, setting and methods, and the main results from included studies. We also extracted data on the type of study, setting, sample source and methods, RT-PCR positive samples for SARS-CoV-2 RNA including cycle threshold (Ct) and copies per m^3, viral culture methods and results, size of air particles (when reported) and proportion in the sample. We tabulated the data and summarised the data narratively by type of sample. Because of substantial heterogeneity across the included studies, we did not perform a meta-analysis. We assessed quality using a modified QUADAS 2 risk of bias tool,[8]. We simplified the tool as the included studies were not designed as primary diagnostic accuracy studies and the quality of transmission studies is known to be low[9]. We gave particular importance to the description of methods for air sampling and the reporting of sufficient detail to replicate. We summarise data narratively and report the outcomes as stated in the paper, including quantitative estimates when reported and the detection of culture of SARS-CoV-2.

Results

We identified 89 studies (see Figure 1; 19 full-text studies were excluded because they were not reviews or there was no SARs-CoV-2 airborne transmission outcome studied and we excluded four laboratory studies: see *Extended data:* Appendix 3 for a list of excluded studies[7]). We included 67 primary studies and 22 systematic reviews (see *Extended data:* Appendix 3 for references to included studies and Table 1 and Table 2 for the characteristics of the included studies[7]).

Reviews

We found 22 reviews on SARS-CoV-2: 16 reviews [Anderson EL 2020, Agarwal 2020, Bahl P 2020, Birgand G 2020, Carducci A 2020, Chen PZ 2020, Comber L 2020, Ekram W 2020, Ji B 2020, Mehraeen E 2020, Niazi S 2020, Noorimotlagh Z 2020, Rahmani 2020, Ren Y 2020, Singhal S 2020, and Wilson NM 2020] were about airborne transmission and prevention; three reviews on airborne transmission and procedures [Hussain A 2020, Kay JK 2020, and Schünemann HJ] and three on ventilation, air conditioning filtration and recirculation [Mousavi EH 2020, Chirico F 2020, and Correia G 2020] (see Table 2). The final search date of these reviews varied from April up to 27 October 2020. Only five reviews met systematic review methods criteria that include systematically searching for all available evidence, appraising the quality of the included studies, and synthesising the evidence into a usable form[10].

Quality of included primary studies (n=67)

All included primary studies were observational (some with experimental components) and low quality (see Table 3). We could not identify a published protocol for any of the studies. Most studies were based on convenience sampling. While the description of methods provided sufficient detail to replicate 91% of studies (see Figure 2), the research often lacked standard methods, standard sampling sizes and reporting. In 69% of the

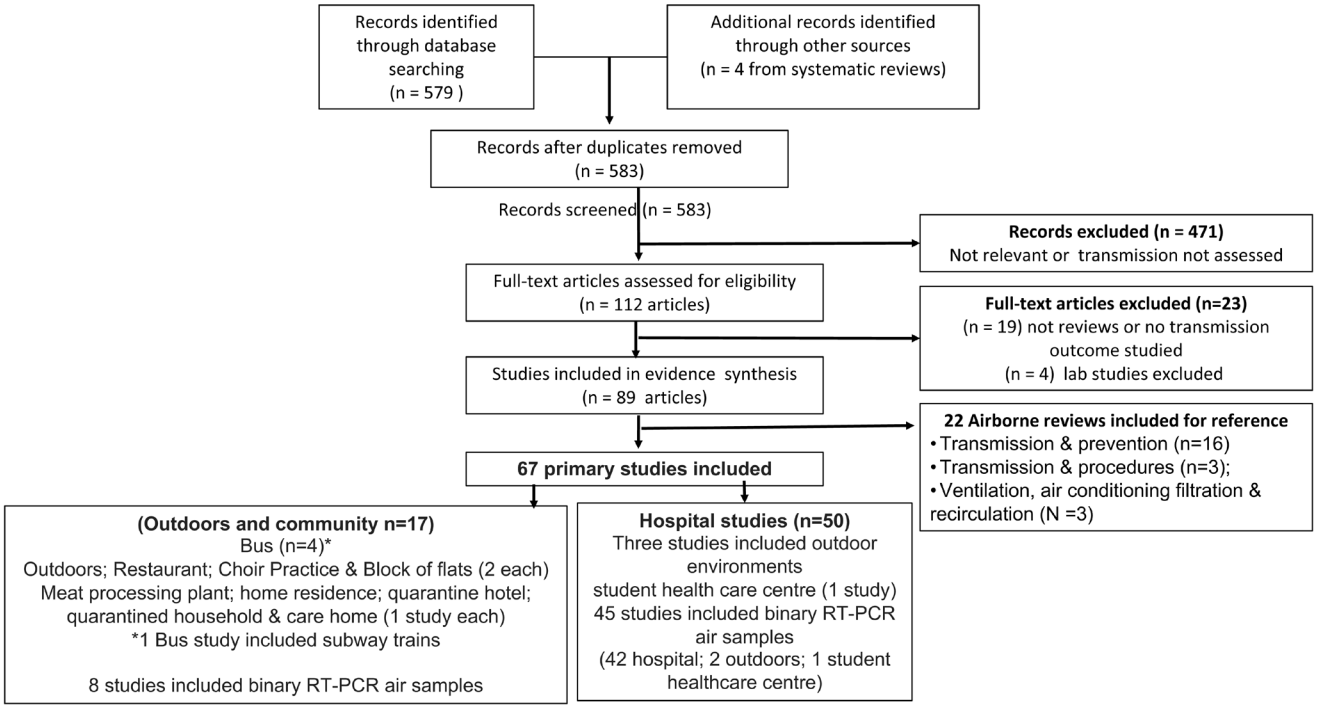

Figure 1. Flow chart.

studies, the sample sources were clear, however, outcomes that aimed to demonstrate the detection of viable, replicable viruses were lacking. Limitations of the sampling methods and the poor-quality reporting make it difficult to discriminate between airborne or droplet nuclei transmission. Interpretation is further limited by the variability in reporting of patient distance from the sampler, use of protective or oxygen masks by patients, patient activities (coughing and sneezing during sampling time), air movement, air conditioning sampler type, sampling, storage and transfer conditions.

Primary studies

We included 67 primary studies, of which 53 (79%) reported binary data on RT-PCR air samples (see Table 1). All were descriptive observational and none were comparative. Twelve studies reported Ct values and 18 report copies per sample volume (see Table 4). Two studies reported a Ct value < 25: Razzini K 2020 *et al.* reports in the intensive care unit the mean Ct was 22.6, and Guo ZD 2020 *et al.* report a Ct of 12.5 near the doctor's office area. Ten studies report Ct values > 35; two [Guo ZD 2020, Lei H 2020] report Ct > 40, and three studies [Dumont-Leblond 2020, Kenarkoohi 2020, Nissen 2020] report the detection of single genes.

Table 5 shows eight studies reporting the size of detectable particles containing RNA [Binder 2020, Chia PY 2020, Chirizzi D 2020, Feng B 2020, Hernández JL 2020, Liu Y & Ning Z 2020, McGain F 2020, and Santarpia 2020a]. Overall the reporting was heterogeneous. SARS-CoV-2 RNA was detectable in a range of air sample sizes from <1 μm through to >18 μm. Seven studies detected particles below <4 μm, and Chirizzi D 2020 *et al.* reported on coarse particles up to diameter > 18 μm. In one study, different samplers detected different size particles: McGain F 2020 *et al.* reported that the APS detected larger aerosols (> 0.37 μm) and MiniWRAS smaller particles (0.01–0.35 μm) (see Figure 3).

We found 36 different descriptions of air samplers deployed: the two most used samplers were the MD8 sampler, Sartorius, Goettingen, Germany (n=7 studies) and the National Institute for Occupational Safety and Health (NIOSH) BC 251 Aerosol sampler (n=6 studies) (see *Extended data:* Appendix 4[7]). One study used four different methods [Ding Z 2020], and in seven studies the sampler used was unclear [Hernández JL 2020, Horve PF 2020, Kang M 2020, Kwon KS 2020, Seyyed Mahdi SM 2020, Tan L 2020 and Zhang D 2020].

Hospital. There were 50 studies conducted in healthcare settings: 45 studies included binary RT-PCR air samples (42 hospitals, 2 outdoors and 1 student healthcare centre).

Of the 42 studies that reported air sampling RT-PCR data within a hospital environment, 24 (57%) reported positive samples (142 positives out of 1,403 samples: average 10.1%). Samples taken per study varied from 2 to 135. In two studies [Ahn 2020 and Santarpia JL 2020b] the denominator was unclear. There was no pattern observed in terms of the type of hospital setting (ICU versus non-ICU) and RT-PCR positivity. Three studies involving ICUs reported 0% of samples were positive [Ma J, Song Z, Wu S] (see Figure 4).

Table 1. Study characteristics: primary studies.

Authors	Setting	Country	Method	Samples source	Air Samples PCR positive for SARs-CoV-2 RNA (unless otherwise stated)	Viral culture	Viral concentrations copies/m3 or copies/L or Cycle threshold (Ct)	size of air particles and proportion in sample	Notes
Ahn JY 2020	Hospital	China	Air (and surface) samples collected. Virus culture was attempted on PCR positive samples.	Air sampling at 1.2 m above floor level, 1.0 m from each patient, using an SKC BioSampler and a Swab sampler.	0/ (denominator unclear) samples	Not attempted.	N/A (no air samples positive)	N/A	Viral RNA was detected in the air outlet fan on the ceiling suggesting airborne contamination by aerosols. Only the outside surface of the endotracheal tubes in the area connected to the ventilator circuit tested positive for SARS-CoV-2 Viable viruses detected on the outside surface of the endotracheal tube and and seven sites in patient 3's room.
Bays D 2020	Healthcare setting	USA	Two detailed case studies	No sampling performed	Not attempted.	N/A	N/A	N/A	A total of 421 health care workers were exposed in total, and the results of the case contact investigations identified 8 secondary infections in health care workers. In all 8 cases, the staff had close contact with the index patients without sufficient personal protective equipment. Importantly, despite multiple aerosol generating procedures, there was no evidence of airborne transmission.
Binder 2020	Hospital	USA	Case series of 20 patients hospitalized with coronavirus disease	8 National Institute for Occupational Safety and Health (NIOSH) BC 251 Aerosol Samplers (Figure S3) were placed 1.5m from the ground, at ~1 meter, ~1.4 meters, ~2.2 meters, and ~3.2 meters from the SARS-CoV-2 patient's head and subsequently run for ~4 hours. 195 air samples were collected	3/195 samples from 3 patients	0/3 viable virus	Sample at 1.4m, <4uM first PCR Ct 36.6, second PCR Ct 37.1 Sample at 2.2m, <4uM first PCR 37.4, second PCR Ct 39.9 Sample at 2.2m, >4uM first PCR 39.1, second PCR Ct 39.6	detected in aerosols particle size <4 µm	for captured droplet size, the NIOSH sampler has roughly a 95% collection efficiency for aerosols with a diameter of 7 µm or less, which decreases t\o approximately 40% efficiency for aerosols ~80 µm in diameter. Dry cyclone aerosol samplers, which are not as well-suited for viable virus collection when compared to liquid collection medium-based bioaerosol samplers
Charlotte N 2020	Choir practice	France	Follow-up of a choir practice: 27 participants, including 25 male singers, a conductor and an accompanist attended a choir practice on 12 March 2020.	No sampling performed	Not attempted.	Not attempted.	N/A	N/A	70% of the participants (19/27) were diagnosed with COVID-19 from 1 to 12 days after the rehearsal (median 5.1 days).
Cheng VCC 2020a	Hospital	China	Air sampling: 6 patients' air sampled, and 5 positive controls	The air sampler was perpendicularly positioned 10 cm away from the patient's chin, collecting at a rate of 50 L/minute. An air tent was used to increase the proportion of exhaled air collected. Participants sneezed directly onto gelatin filter and spit saliva droplets onto gelatin filter.	0/6	Not attempted.	N/A (no air samples positive)	N/A	Infection isolation rooms had 12 air changes per hour. 5% surface samples were found to be PCR-positive.
Cheng VCC 2020b	Hospital	China	Air sampling using ISO 180 model 86834 air sampler was performed in the room of a patient.	Air samples were collected 10 cm from the one patient's chin. The patient performed 4 different manoeuvres (normal breathing, deep breathing, speaking "1, 2, 3" continuously, and coughing continuously) while putting on and removing the surgical mask.	0/8	Not attempted.	N/A (no air samples positive)	N/A	SARS-CoV-2 was identified in 1 of 13 environmental surface samples of the patient's room.

Authors	Setting	Country	Method	Samples source	Air Samples PCR positive for SARs-CoV-2 RNA (unless otherwise stated)	Viral culture	Viral concentrations copies/m3 or copies/L or Cycle threshold (Ct)	size of air particles and proportion in sample	Notes
Chia PY 2020	Hospital	Singapore	Air (and surface) sampling surrounding 61 hospitalized COVID-19 patients in airborne infection isolation rooms	Air sampling was performed in three of the 27 airborne infection isolation rooms (AIIRs). Bioaerosol samplers used to collect air samples, set at a flow-rate of 3.5 L/min and run for four hours, collecting a total of 5,040 L of air from each patient's room.	2/3	Not attempted.	Total SARS-CoV-2 concentrations in air ranged from 1.84×103 to 3.38×10^3 RNA copies per m^3 air sampled.	positive particles of sizes >4 μm and 1–4 μm detected in two rooms	Patient 1 intermittently faced the samplers seated 1m from the 1st tripod and 2.1m from the 2nd. In the rooms of Patients 2 and 3, three NIOSH samplers were attached to each of two tripod stands at 1.2m, 0.9m, and 0.7m height. Patients 2 & 3 remained in bed within 1 m from all 6 air samplers. Patient 3 was also talking on the phone for much of that time.
Chirizzi D 2020	Outdoor	Italy	Study of the outdoor concentrations and size distributions of virus-laden aerosol simultaneously collected, in May 2020, in northern (Veneto) and southern (Apulia) regions of Italy.	Genetic material of SARS-CoV-2 (RNA) was determined, using both real time RT-PCR and ddPCR, in air samples collected using PM10 samplers and cascade impactors able to separate 12 size ranges from nanoparticles (diameter D & 0.056 μm) up to coarse particles (D > 18 μm).	Outdoor atmospheric concentrations of SARS-CoV-2 were very small (<0.8 copies m–3)	Not attempted.	SARS-CoV-2 concentrations were <0.8 copies m–3 for each size range.	(D < 0.056 μm) up to coarse particles (D > 18 μm)	It is possible to conclude that outdoor air in residential and urban areas was generally not infectious and safe for the public in both northern and southern Italy, with the possible exclusion of very crowded sites (See Liu 2020)
Declementi M 2020	Hospital	Italy	Air sampling to assess environmental contamination in a COVID-19 non-Intensive Care Unit. Two patients admitted to the hospital rooms were positive for COVID-19 for more than a week.	8 air samples were collected before and after the application of two different sanitization devices. Pumps were placed in 4 sites: patient 1 room, patient 2 room, an empty room nearby patients' rooms, corridor outside the rooms. Pumps (47 mm filter cassettes and 0.45 μm filters in polytetrafluoroethylene-PTFE) positioned 1 meter above the floor for 340 minutes at 15 l/min.	0/8	Not attempted.	N/A (no air samples positive)	N/A	Surface samples collected before and after sanitization (n=24) were all negative. Patient 1 rhinopharyngeal swab was positive while patient 2 rhinopharyngeal swab was negative (Table 2).
De Man P 2020	Care home	The Netherlands	Case series. Responding to an outbreak in a care home, the ventilation system of the outbreak ward was investigated in addition to routine source and contact tracing	No air samples collected.	Not attempted.	N/A	N/A	N/A	The ventilation system allowed recirculation of air below a certain CO2 limit. The outbreak ward was additionally cooled by 2 air conditioning units, which recirculated air through a 1-mm mesh dust filter. The other 6 wards (no outbreak) were ventilated with outside air.
Di Carlo P 2020	Inside a bus	Italy	Observational measurements were carried out across the last week of the lockdown and the first week when, gradually, all travel restrictions were removed. 12 to 22 May 2020 in Chieti, Italy.	Samples of air inside the bus were taken every day of the two observational weeks, excluding weekends. Two microbiological gelatine membrane sample filters of 80 mm diameter were installed on board: one close to the ticket machine, the other on the rear part of the bus. All the air samples were gathered during the 6.5 hours daily operation of the bus,	0/14	Not attempted.	N/A (no air samples positive)	N/A	During the whole observation period about 1100 passengers travelled on the trolleybus. Hand sanitizing was a strict requirement to get on the bus, and rule of wearing a facial mask during travel, and the recommendation to keep the windows open to allow high air ventilation.
Ding Z 2020	Hospital	China	Sampling, including of air, within and around 4 isolation rooms each with 3 patients. Other areas in the hospital and its roof air-exhausts were also sampled.	46 air samples, two exhaled condensate samples, and two expired air samples (also 47 surface samples) were collected within and beyond the 4 three-bed isolation rooms.	1/46 air samples weakly positive. Both exhaled condensate samples negative. Both expired air samples negative.	Not attempted.	RNA copies for weakly positive sample not calculated.	NR	The toilet area was the area most contaminated by SARS-CoV-2 RNA as detected by PCR. Two exhaled condensate samples and the two expired air samples were negative .
Dohla M 2020	Quarantined households	Germany	Study of 43 adults and 15 children living in 21 households; air (also surface and wastewater) samples taken.	Air samples obtained using Coriolis Micro-Air sampler; air collectors were positioned in the middle of the room used most frequently by the residents (usually the living room or kitchen) - no rooms had ventilation equipment. Close contact to the air sampler was avoided (e.g. speaking in a range below 2 m but not above 3 m).	0/15	Infectious virus could not be isolated in Vero E6 cells from any environmental sample.	N/A (no air samples positive)	N/A	26 of all 43 tested adults were positive by RT-PCR. 10 of 66 wastewater samples and 4/119 surface swab samples were positive for SARS-CoV-2

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Authors	Setting	Country	Method	Samples source	Air Samples PCR positive for SARs-CoV-2 RNA (unless otherwise stated)	Viral culture	Viral concentrations copies/m3 or copies/L or Cycle threshold (Ct)	size of air particles and proportion in sample	Notes
Dumont-Leblond N 2020	Hospital	Canada	Air sampling in acute care hospital rooms over the course of nearly two months	100 air samples in acute care hospital rooms hosting 22 patients using three different air sampling protocols. Two conductive plastic Institute of Occupational Medicine (IOM) samplers with 3 µm gelatine filters or one IOM and a 37 mm cassette with 0.8 µm polycarbonate filters.	11/100 from 6 patient rooms	Viral cultures were negative	Among 11 positives for N gene target, Ct ranged from 36.46 to 39.8, mean 37.99. Among 8 positives for ORF1b target, Ct ranged from 32.07 to 35.15, mean 33.69 Among the 8 positive for both N and Orf1b, viral concentrations ranged from 9.86 to 514.17, mean 201.64 genomes /m^3	NR	Even when the IOM and cassette were positive (patient D), SARS-CoV-2 could not be recovered in the air using the SASS®3100 according to the protocol. 7/11 positive air samples collected using IOMs. When both IOMs and cassettes were used , cassettes lead to positive results more often than IOMs (5/5 vs. 3/5) and higher concentrations overall. "No live virus was isolated from air samples, either due to viral inactivation through the sampling process or the true absence of whole, infectious virions."
Faridi S 2020	Hospital	Iran	Air sampling in wards of Covid-19 patients with severe and critical symptoms.	10 air samples were collected into the sterile standard midget impingers containing 20 mL DMEM with 100 µg/mL streptomycin, 100 U/mL penicillin and 1% antifoam reagent for 1 h. Air samplers placed 1.5 to 1.8 m above the floor and approximately 2 to 5 m away from the patients' beds. Some patients coughed during the sample collection.	0/10	Not attempted.	N/A (no air samples positive)	N/A.	Air samples which were collected 2 to 5 m from the patients' beds
Feng B 2020	Hospital	China	Environmental contamination investigated around 21 COVID-19 patients in the later stage of infection	For sampling of isolation room air, a NIOSH sampler was placed on a tripod 1.2 m in height and 0.2 m away from the bed at the side of the patient's head. The sampling duration was 30 min, and a total of 105-L room air was sampled. (9 Exhaled Breath (EB) samples, 8 Exhaled Breath Condensate (EBC) samples, 12 bedside air samples)	0/14 EB 2/8 EBC 1/12 room air	Not attempted.	RNA detected in air sample, with virus concentrations of 1111 copies/m^3 and 744 copies/m^3 in the <1 µm and >4 µm fractions, respectively	RNA detected in air sample in <1 µm and >4 µm fractions,	An exhaled aerosol collection system was developed and the patients were asked to breathe normally through a mask for 30 min, and asked to perform 10 forced coughs during this time.
Ge XY 2020	Hospital	China	Environmenta; air samples from 6 different sites of 3 hospitals	Air samples were collected for 30 min using the National Institute for Occupational Safety and Health (NIOSH) bioaerosol sampler (BC251) with air pumps (XR5000, SKC). The stream of air has been set to 3.5 L / minute.	ICU 3/3 Haemodyalysis clinic 0/12 fever clinic 0/12 respiratory ward 0/6	Not attempted.	ICU: Ct 36.5 - 37.8	NR	
Günther T 2020	Meat Processing Plant	Germany	Staff tested based on self-reported symptoms, possible contacts to other infected persons, returning to work after more than 96 h absence from work	Eight air conditioning units placed near the ceiling in the proximal half of the room constantly cool the air. Fans project the air in a lateral direction, either directly from frontal openings in the unit or via perforated hoses mounted underneath the ceiling	Not attempted	Not attempted.	N/A.	N/A.	Low temperature, low air exchange rates, and constant air recirculation, together with relatively close distance between workers and demanding physical work, may have promoted efficient aerosol transmission.
Guo ZD 2020	Hospital	China	Air (and surface) samples of ICU and Covid-19 wards.	Indoor air and the air outlets were sampled to detect aerosol exposure. Air samples were collected by using a SASS 2300 Wetted Wall Cyclone Sampler at 300 L/min for 30 min. Samples were tested for the open reading frame 1ab and nucleoprotein (N) genes of SARS-CoV-2 by qRT- PCR	AIr samples: 14/40 ICU* 2/16 General Ward Air outlet swab samples: 8/12 for ICUs 1/12 for GWs.	Not attempted.	Indoor air near the air outlet: Ct 35.7, 3.8/L Indoor air near the patients: Ct 44.4. 1.4/L Indoor air near the doctor's' office area: Ct 12.5 0.52/L	NR	*ICU high-risk area was the patient care and treatment area, where rate of positivity was 40.6% (13/32). The low-risk area was the doctor's' office area, where rate of positivity was 12.5% (1/8).
Hamner L 2020 and Miller SL 2020	Choir Practice	USA	Follow up of choir practice attendees	In total, 78 members attended the 3rd March 2020 practice, and 61 attended the 10th March 2020 practice. Overall, 51 (65.4%) of the 3rd March practice attendees became ill; all but one of these persons also attended the 10th March practice. Among 60 attendees at the 10th March practice (excluding the patient who became ill 7th March, who also attended), 52 (86.7%) choir members subsequently became ill. 32 were confirmed and 20 probable secondary COVID-19 cases occurred.	Not attempted.	Not attempted.	N/A	N/A	Attendees had a 15-minute break, during which cookies and oranges were available at the back of the large room. No one reported physical contact between attendees. The seating chart was not reported because of concerns about patient privacy. One hour of the practice occurring outside of the seating arrangement,

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Hernández JL 2020	Hospital	Mexico	Air samples of Emergency areas and Covid-19 patients rooms.	Air sampled in three areas: Emergency area (Clinic A), Internal medicine (Clinic A), COVID 19 patient area (Clinic A), and COVID-19 patients care room (Clinic B). Sampling in all areas was accomplished in 3 h. Filters of 25 mm diameter with 0.22 µm pores were utilized (Millipore, AAWP02500), placed in a sterilized filter holder (Millipore, SWINNX) coupled to a vacuum system through a previously disinfected plastic hose, filtering the air with a flow of 9.6 L/min in each filter holder.	3/15	Not attempted.	Not reported	filtration through 0.22 µm pores.	All three positive samples were in COVID 19 patient area (Clinic A)
Horve PF 2020	Hospital	USA	Air handling units (AHUs) sampled, including the pre-filters, final filters, and supply air dampers.	Samples were collected using Puritan PurFlock Ultra swabs and swabs were taken in triplicate at each AHU location from the left, middle, and right side of each area along the path of airflow. Swabs were pre-moistened using viral transport media. Swabbing occurred for 20 seconds on an area approximately 20 X 30 cm at each location and swabs were immediately placed into 15 mL conical tubes (Cole-Parmer, catalog #UX-06336-89) containing 1.5 mL viral transport media and stored on ice for transport to a BSL-2 laboratory with enhanced precautions (BSL2+) lab for processing, which typically occurred within two hours after collection.	14/56 s	Not attempted.	The highest abundance sample (~245 gene copies) was found on the pre-filters, where outside air mixes with recirculated building air. [Other copy number data not found in report.]	NR	Highest abundance sample (~245 gene copies) was found on the pre-filters, where outside air mixes with recirculated building air. Of the samples collected, 35% (7/20) of samples at the pre-filters, 16.67% (2/12) of samples at the final filter, and 20.8% (5/24) of samples at the supply air dampers contained detectable SARS161 CoV-2 RNA
Hu J 2020	Hospital	China	Indoor and outdoor air samples in ICUs and CT rooms	Aerosol samples were collected over 30 min intervals with the use of a centrifugal aerosol-to-hydrosol sampler (WA-400, Beijing Dingblue Technology Co., Ltd., China). Twenty-three masks from patients and 24 swabs from surfaces in ICUs were also collected and analyzed. Ten 3M™ Versaflo™ TR-600 respirator filters and 40 masks from healthy workers in the P3 lab of Wuhan Institute of Virology were collected for viral RNA detection. The airflow rate of the respiratory filters was 190 L/min and the surface area was ~30 cm2. All viral RNA positive aerosol samples were subjected to cell culture. All viral RNA positive aerosol samples were subjected to cell culture to determine whether viable virus could be recovered from them.	Aerosol samples 8/38 from ICUs 1/6 from CT rooms samples from medical staff rest areas and corridors, were all negative (denominator not clear)	All positive aerosol samples were negative after three passages of Vero-E6 cells inoculated in a blind test.	The range of virus concentrations in the positive aerosol samples was 1.11 × 10^3 to 1.12 × 104 RNA copies m^3 . In 10% of the outdoor air samples collected 10 m from the doors of inpatient and outpatient buildings, respectively, viral concentrations ranged from 0.89 to 1.65 × 10^3 RNA copies m^3.	NR	5 surface swabs (cabinet, patient's bed rail, door handle, and patient monitor) out of 24 from the ICU were positive for SARS-CoV-2, with viral RNA. After rigorous disinfection, no viral RNA was detected in a second batch samples from the same places. Positive rates for the mask samples were relatively high compared with the aerosol or surface samples. All positive masks were subjected to cell culture and inoculated with Vero-E6 cells after blind passage for three generations. One mask from a critically ill patient detected positive.
Jiang Y 2020	Hospital	China	Indoor air samples from Covid-19 isolation ward	Air was collected by two methods: natural sedimentation and a microbial air sampler (MAS-100 ECO), for which the stream of air was set to exactly 100 liters/minute (Merck, Germany).	1/28 air samples	Not attempted.	Not reported	NR	The isolation ward with an ICU patient was positive. Based on the original 24 hours of UV air filtering and 1000-2000 mg/L chlorine-containing disinfectant for ambient air and floor disinfection, the frequencies and duration times of air disinfectants were extended. Key surfaces such as computer keyboards easily overlooked were clearly noted and carefully disinfected. The samples from the positive area and indoor air were collected 24 hours later, and the test results were negative.
Jin T 2020	Hospital	China	Air and surface samples of ICU of one Covid-19 patient.	Two hours after routine cleaning, high-volume air samples were taken 0.5m from the patient bed and in the staff PPE dressing room, using a WA 400 Portable viral aerosol sampler at 400 L/min for 15 min at 1.5m height, while the patient was present and was not wearing a mask.	Air sample: 0/1 staff PPE dressing room 1/1 ICU patient isolation room	Not attempted.	Not reported	NR	All surface samples tested negative. The concentration of airborne SARS-CoV-2 was not quantified. The aerodynamic size distribution of SARS-CoV-2 aerosols was not evaluated.

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Kang M 2020	Block of flats	China	Air (and surface) sampling, and experimental air flow study.	Air samples from 11 of the 83 flats in the building, public areas, and building drainage systems.Investigated gas flows and dispersion as an indicator of the movement of virus-laden droplets in the drainage system, tracer gas (ethane) was released into bathrooms. The hydraulic interactions of toilet wastewater and the stack were observed.	0/11 air samples	Not attempted.	N/A (no air samples positive)	N/A	Virus-containing fecal aerosols may have been produced during toilet flushing by index cases. The infected families lived in 3 vertically aligned flats connected by drainage pipes in the master bathrooms. There were 9 infected patients, 193 other residents of the building, and 24 members of the building's management staff.
Kenarkoohi A 2020	Hospital	Iran	Air sampling through hospital wards indoor air by confirmed COVID-19 patients on 7th May 2020.	A liquid impinger biosampler calibrated for a flow rate of 12 L.min□1 at 1.5 m above ground floor and at least 2 m away from the patient beds was used to take fourteen air samples in different wards of the indoor air of the hospital: ICU, ICU entrance hall, hospital entrance hall, laboratory ward, CT scan, radiology, men internal ward, woman internal ward and emergency ward.	2/14 air samples (both in ICU)	Not attempted.	Cycle threshold (CT) values were around 38 and 35 for ORF1ab and nucleoprotein gene, respectively.	The particulate matter PM1, PM2.5 and PM10 concentrations during the air sampling in the hospital wards was reported but no RNA samples	Two of 14 air samples contained SARS-CoV-2 RNA. Droplet vs airborne could not be distinguished.
Kim UJ 2020	Hospital	Korea	Surface and air sampling.	The rooms of 8 COVID-19 patients in four hospitals. On days 0, 3, 5, and 7 of hospitalization, the surfaces in the rooms and anterooms were swabbed, and air samples were collected 2 m from the patient and from the anterooms.	0/52 air samples positive for SARS-CoV-2 RNA	Not attempted.	N/A (no air samples positive)	N/A	All 52 air samples from 8 Covid-19 patients' rooms were negative for SARS-CoV-2 RNA. Surface contamination with SARS-CoV-2 RNA was widespread; negative after disinfectant cleaning.
Kwon KS 2020	Community	Korea	Investigation was implemented based on personal interviews and data collection on closed-circuit television images, and cell phone location data.	A total of 39 environmental samples of inlets and outlets of air conditioners, table seat of case A, and nearby tables and chairs in consideration of air flow direction were collected on June 23 for testing of SARS-CoV-2 in the environment and were analyzed by rRT-PCR test. Air speed and direction at several specified positions were precisely measured using a portable anemometer	0/39 positive	Not attempted.	N/A (no air samples positive)	N/A	Maximum air flow velocity of 1.2 m/s was measured between the infector and infectee in a restaurant equipped with ceiling-type air conditioners. Environmental samples were collected at 11 days after the inspector visit.
Lednicky JA 2020a	Hospital	USA	Air samples collected, and virus culture attempted	VIVAS air samples from the room of two COVID-19 patients were set up 2m to 4.8m away from the patients. Three serial 3-hr air samples were collected. For each sampler, the second of the three samplings was performed with a high efficiency particulate arrestance (HEP A) filter affixed to the inlet tube, a process to reveal whether virus detected in consecutive samplings reflect true collection and not detection of residual virus within the collector.	4/4 air samples without a HEPA filter 0/2 samples using a HEPA filter	Virus-induced CPE were observed for 4/4 RNA-positive air samples.	Four positive samples estimated to contain: 2.82E+03, 9.12E+02, 1.15E+03, 4.68E+02 genome equivalents/ 25 µL, with Cycle quantification (Cq) values 36.02, 37.69, 37.42, 38.69, respectively (mean Cq 37.46)	NR	No other respiratory virus was identified in the samples using a BioFire FilmArray Respiratory 2 Panel. The amount of airborne virus detected per liter of air was small. Plaque assays could not be performed due to a nationwide non availability of some critical media components (due to COVID-19 pandemic-related temporary lockdown of production facilities), so TCID50 assays were performed in Vero E6 cells to estimate the percentage of the collected virus particles that were viable. Estimates ranged from 2 to 74 TCID50 units/L of air
Lednicky JA 2020b	Student Healthcare centre	USA	Air samples collected, and virus culture attempted	The air sampling device was placed in a hallway along which potential Covid-19 cases walked, wearing a mask, to reach clinical evaluation rooms. The air inlet was approximately 1.5m above floor level.	1/2	General virus-induced cytopathic effects were observed within two days post-inoculation	0.87 virus genome equivalents L -1 of air; Ct value 39.13	NR	The amount of virus present in 390 L of sampled air was low (approximately 340 virus genome equivalents). PCR tests for SARS-CoV-2 vRNA from cell culture were negative. Three respiratory viruses were identified using the Biofire RVP: Influenza A H1N1, Influenza A H3N2, and Human coronavirus OC43

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Lei H 2020	Hospital	China	Air and surface samples from the intensive care unit (ICU) and an isolation ward for COVID-19 patients.	Air samples were collected with a two-stage cyclonic bioaerosol sampler (NIOSH) and an aerosol particle liquid concentrator, between 8am and 12 noon. The NIOSH sampler was placed on a tripod at the head of the bed within 1m of the patient's head at a height of 1.3 m. In the isolation ward, the sampler was also used in the bathroom by mounting it on an infusion support near the sink, < 1m from the toilet.	Surface and air: 1/218 ICU samples 2/182 isolation ward samples	Not attempted.	near the head of the patient Ct 41.25.	NR	The number of air samples is unclear. One air sample collected using the DingBlue sampler placed near the head of the patient in bed 14 showed amplification at cycle threshold (Ct) 41.25. The detection of viral RNA was also in the air samples in the bathroom.
Li YH & Fan YZ 2020	Hospital	China	Aerosol samples & surface samples collected in a hospital for severe COVID-19 patients	Aerosol samples collected by an impingement air sampler BIO-Capturer-6. 135 135 aerosol samples from 45 locations taken from the ICU ward, general isolation wards, fever clinic, storage room for medical waste, conference rooms and the public area.	0/135	Not attempted.	N/A (no air samples positive)	N/A	The ICU ward has 12 air inlets with 16 discharges per hour; isolation room 8 air inlets with 12 discharges per hour. COVID-19 patients in a ward were separated by a minimum of 1.5 m. Air was cleaned with 4-time-daily air disinfection using a plasma air steriliser. with the exception of two samples from a COVID-19 patient's mask, all 90 environmental surfaces sampled were negative for SARS-CoV-2.
Li Y & Qian H 2020	Restaurant	China	Observational and experimental: Data from a video record and a patron seating-arrangement from the restaurant in Hong Kong were collected. Secondly, the dispersion of a warm tracer gas was assessed, as a surrogate for exhaled droplets	No sampling performed	Not attempted.	N/A	N/A	N/A	
Lin G 2020	Block of flats	China	Case series: Nine COVID-19 cases in one community in Guangzhou who lived in three vertically aligned units of one building sharing the same piping system.	Given that all the cases occurred in the same unit and that these households shared a common pipe system, we therefore conducted a tracer-gas experiment to simulate the process of potential transmission through air	Not attempted.	N/A	N/A	N/A	Airflow detection and simulation experiment revealed that flushing the toilets could increase the speed of airflow in the pipes and transmitted the airflow from Apartment 15-b to 25-b and 27-b. Reduced exhaust flow rates in the infected building might have contributed to the outbreak.
Liu Y & Ning Z 2020	Hospital and public spaces	China	Measured SARS-CoV-2 RNA in air samples from 2 Covid-19 hospitals, and quantified the copy counts using a droplet digital PCR-based detection method	Over a 2 week period: 30 aerosol samples of total suspended particles collected on 25-mm-diameter filters loaded into styrene filter cassettes (SKC) by sampling air at a fixed flow rate of 5.0 l min−1 using a portable pump (APEX2, Casella). Three size-segregated aerosol samples collected using a miniature cascade impactor (Sioutas Impactor, SKC) that separated aerosols into five ranges (>2.5 μm, 1.0 to 2.5 μm, 0.50 to 1.0 μm and 0.25 to 0.50 μm on 25-mm filter substrates, and 0 to 0.25 μm on 37-mm filters) at a flow rate of 9.0 l min−1. Two aerosol deposition samples collected using 80-mm-diameter filters packed into a holder with an effective deposition area of 43.0 cm2; filters were placed intact on the floor in two corners of an ICU for 7 days.	ICU, 2/3 positive 15/22 Isolation wards & ventilated rooms 4/11 public areas	Not attempted.	ICU - 31 &113 copies m^3 Isolation wards & ventilated rooms (concentrations very low: <43 m^3. public areas (very low concentrations (<11 m^3)	NR	Negatively pressurized isolation and high air exchange rates were inside the intensive care units, coronary care units and ward room. Reported values are virus aerosol deposition rates in copies m−2 h−1. Very low or undetectable concentrations of airborne SARS-CoV-2 were found in most of the patient areas of Renmin Hospital. The authors suggest that the negatively pressurized isolation and high air exchange rate inside the intensive care units, coronary care units and ward room of Renmin Hospital are effective in limiting the airborne transmission of SARS-CoV-2.
Lu J 2020	Restaurant	China	Study of an outbreak apparently centred on a restaurant; air flow studied & surface samples taken	Air samples not taken. 6 smear samples taken from the air conditioner (3 from the air outlet and 3 from the air inlet)	Not attempted.	N/A	N/A	N/A	0/6 smear samples tested positive by PCR.

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Luo K 2020	Bus trip	China	Case study of a SARS-CoV-2 outbreak event during bus trips of an index patient in Hunan Province, China.	No sampling performed	Not attempted.	N/A	N/A.	N/A.	Could not verify transmission via fomites as no environmental samples were collected; (2) the SAR was likely overestimated as it is solely based on a single large cluster; (3) there might be recall bias because the information (including the seat number) was collected retrospectively; (4) no viral genetic sequence data were available from these cases to prove linkage; and (5) some of the secondary and tertiary cases could have been exposed to unknown infections
Ma J 2020	Hospital and quarantine hotel	China	Exhaled breath condensate (EBC) samples were collected from 20 imported COVID-19 cases, 29 local cases and 15 healthy controls.	EBC samples were collected using a BioScreen device developed by Peking University. 242 surface swabs from quarantine hotels and hospitals or from personal items of COVID-19 patients were obtained using wet cotton swabs	14/52 EBC sample positive; 1/26 air samples positive	Not attempted.	EBC samples, 14 positive: Ct values 35.54 ± 3.14 were obtained for each positive. The breath emission rate was estimated to be from 1.03 × 10 5 to 2.25 × 10 7 viruses per hour. The positive air sample was estimated to contain 6.07 × 10 3 viruses/m3.	NR	In the ward of patient C, the virus was present on the surface of an air ventilation duct entrance that was located below the patient's bed. Cycle threshold range (N or ORF1a/b) for EBC samples 35.5 ± 3.1 and air samples CT = 38.4. 1 sample (air-1) from an unventilated quarantine hotel toilet room was positiv SARS-CoV-2 emission does not continue at the same rate but rather is a sporadic event. For example, 2 EBC samples (EBC-1, EBC-2) collected from patient E but on different dates and using the same method returned different test results
Marchetti R 2020	Hospital	Italy	Air sampling in three different hospitals in Milan, Italy.	For particles' sampling the AEROTRAK™ Portable Airborne Particle Counter was used for cleanroom particles classification. For microbiological air sampling, the SAS Super IAQ Surface Air System (model 90593), which conveys a known volume of air during a fixed period on Petri Plates filled with Standard Plate Count Agar (PCA) was used. Ten AIRcel units per hospital were placed in three different hospitals in Milan, Italy. In total 68 samples were processed in three distinct test sessions between April and June 2020, using the QIAGEN Rotor-Gene thermal cycler.	E gene 19/68 samples, ORF1ab + N detected in 7/68 samples. .	Not attempted.	Not reported	NR	The result of the RT-PCR showed a marked presence of the target β-coronavirus E gene for 19 of the 68 samples, while the target ORF1ab + N was detected in 7 samples. In particular, at Sacco Hospital, the test results show the detection of ORF1ab + N and/or E gene in 15 samples out of 40.
Masoumbeigi H 2020	Military hospital	Iran	Random air sampling with continuously sterilised sample equipment	All patients aged 55-65 were either intubated or had severe symptoms. Sampling of 100-1000 l for 20 mins in two randomly chosen stations 0.5 metres from the beds. RT-PCR performed at 42 cycles. Air sampling was done (n= 31) on selected wards including Emergency 1, Emergency 2, bedridden (4-B, 10-D), ICU 2, ICU 3, CT-SCAN, and laundry.	0/31	Not attempted.	N/A (no air samples positive)	N/A.	Cycle threshold for detection used = 42
McGain F 2020	Hospital	Australia	Case report of a tracheostomy procedure; air samples were collected throughout	Two spectrometers to measure aerosol particles: the portable Mini Wide Range Aerosol Sizer 1371 (MiniWRAS) and the Aerodynamic Particle Sizer (APS). During the procedure, the aerosol detector inlet was positioned 30 cm directly above the patient's neck, representing the surgeon's breathing air space	Not attempted.	Not attempted.	N/A	APS detected larger aerosols (> 0.37 mm) and MiniWRAS smaller particles (0.01–0.35 mm).	Maximum aerosol particles were generated during diathermy, but overall low levels. It is unlikely that viruses will survive the high temperature of diathermy.

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Moreno T 2020	Buses and Subway Trains	Spain	75 samples from buses and 24 from subway trains, collected from surfaces using swabs (78 samples), from ambient air (12 samples), and from air-conditioning filters (9 samples)	Air sampling in the subway took place June 17–19, 2020 on three consecutive days. Six samples of particulate matter with a diameter of <2.5 µm (PM2.5) were collected inside 6 trains using 47 mm Teflon filters with PEM (Personal Environmental Monitor) equipment. The sampling of the buses took place between 20:00 and 03:00 on the night of May 25–26, 2020 in one of the four main bus depots in Barcelona. After sampling, the bus was disinfected.	1/6 air samples on buses gave weak positive result 2/6 subway trains	Not attempted.	Bus: Positive sample genome count values ranged between 14 to 446/m2 for IP2, 9 to 490/m2 for IP4 and 5 to 378/m2 for E. Subway: first positive sample estimated viral load 23.4 GC/m3, second positive sample the amplified target gene regions were IP2 (18.8 GC/m3) and the envelope protein E (5.6 GC/m3).	NR	Only fragments of 1 or 2 gene targets were identified, not an infectious virion, and furthermore, even if they were infectious viruses, it is estimated that only one particle in 10 million would be able to produce an infectious cycle. Bus calculated viral load (supposing that RNA represents the unlikely worst situation of being representative of infective virus load) of 1.44 GC/m3. 6 PM2.5 samples collected in ambient air 2 gave a positive signal . In the first case the target gene region identified was IP2, with a figured viral load of 23.4 GC/m3. In the second case the amplified target gene regions were IP2 (18.8 GC/m3) and the envelope protein E (5.6 GC/m3)
Morioka S 2020	Hospital	Japan	2 case reports	Air was sampled using an MD8 airscan sampling device and sterile gelatin filters. Air was sampled twice at a speed of 50 L/minute for 20 minutes in the negative-pressure rooms of two patients and its associated bathrooms.	0/2 patient 1 0/2 patient 2	Not attempted.	N/A.	N/A.	Intubation was performed for patient 1 three days before the sampling. Patient 2 was asymptomatic when the sampling was conducted.
Mponponsuo K 2020	Hospital	Canada	Epidemiological study investigating airborne versus droplet transmission of SARS-CoV-2	Air samples not taken. From 5 HCWs with positive SARS-CoV-2 tests and Covid-19 symptoms, no onward transmission was observed from 72 exposures	Not attempted.	Not attempted.	N/A.	N/A.	No transmission from multiple high-risk exposures from 5 COVID-19 HCWs to either patients or other staff.
Nakamura K 2020	Hospital	Japan	Nasopharyngeal, environmental and air samples from patients	11 air samples in three negative pressure bays (Bay 1 to Bay 3), a single negative pressure room in a general ward (Room 1) and a single negative pressure room in an isolation ward (Room 2) using an MD8 airscan sampling device (Sartorius, Goettingen, Germany) and sterile gelatin filters (80 mm diameter and 3 µm pores; Sartorius). We placed the device on the floor about 1.5 meters–2 meters away from the patient's head. Air was sampled twice, at a speed of 50 L/minute for 20 minutes, in the negative pressure rooms and its associated restrooms	0/11	Not attempted.	N/A (no air samples positive)	N/A (no air samples positive)	4/141 swab samples collected from the three bays and two single rooms were positive
Nissen K 2020	Hospital	Sweden	Observational: surface swabs and fluid samples collected, and experimental: virus culture was attempted.	In a Covid-19 ward, surface samples were taken at air vent openings in isolation rooms and in filters. Fluid sample collections were done in the ventilation system.separate HEPA filter systems, distance measured to between 49 and 56 meters. Admitted patients in the ward were between day 5 and 23 after symptom onset	7/19 room vents 11 days later, 4/19 for both genes. 8/9 main exhaust filters +ve for both genes.	No significant CPE was seen after three passages on Vero E6 cells from samples retrieved from ward vent openings or central ventilation ducts or filters	Petri dishes Ct values 35.32 and 33.16 for N and E genes respectively. Ward 1 +ve specimen had Ct value 33.00, for E gene only.	NR	Cycle threshold (Ct) values varied between 35.3 and 39.8 for the N and E gene. Virus culture was attempted: RNA detected in sequential passages but CPE not observed. Petri dishes of cell medium, placed in inspection hatches in the central ventilation system prior to the exhaust filters, were +ve for both N and E genes. 1/3 specimens from ward 1 contained only the E gene.

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Ogawa Y 2020	Hospital	Japan	Observational study of 15 HCP who had contact exposures (15/15) and aerosol exposures (7/15) to a hospitalized Covid-19 patient who re-tested positive 18 days after initial negative PCR.	Air sampling not performed, All PCR tests performed on exposed HCWs using a nasopharyngeal swab obtained on the 10th day after the exposure were negative, and the results of the tests for IgG antibodies to SARS-CoV-2 on the specimens collected approximately 20 days after exposure were also negative.	Not attempted.	N/A	N/A.	N/A	Due to sudden change in symptoms, the patient underwent nasopharyngeal and sputum PCR testing for SARS-CoV-2 again; the results were re-positive. Due to this, several HCP who had interacted with this patient had to be marked for active isolation. The core issue is determining whether the patients whose PCR test results are re-positive are infectious. A low probability of infection from a re-positive case.
Ong SWX 2020	Hospital	Singapore	Air, surface and PPE swab samples collected for 3 hospitalized Covid-19 patients.	Air sampling was done on 2 days using SKC Universal pumps (with 37-mm filter cassettes and 0.3-μm polytetrafluoroethylene filters for 4 hours at 5 L/min) in the room and anteroom and a Sartorius MD8 microbiological sampler (with gelatin membrane filter for 15 minutes at 6 m3/h) outside the room. Supplemental file Blue icons labelled A to E indicate the position of the air samplers within the room (A to C), anteroom (D), and common corridor (E).	0/5	Not attempted.	N/A (no air samples positive)	N/A	Surface contamination with SARS-CoV-2 RNA was widespread but undetectable after cleaning; swabs taken at air outlets tested positive suggesting droplet deposition; all air samples were negative.
Orenes-Piñero E 2020	Hospital	Spanish	Study of COVID-19 traps to measure the capacity of SARS-CoV-2 aerosol transmission.	"COVID-19 traps"were placed only in the rooms of patients with a confirmed positive diagnostic. Interestingly, the rooms where COVID-19 patients were isolated had a ventilation rate of 1800 m3/h. 6 different surfaces trapped in boxes with plastic, protective grids to avoid that samples could be touched by the patient or by the healthcare personnel. The different surfaces were: polypropylene (PP), glass, polyvinyl chloride (PVC), methacrylate, agar medium and carbon steel. PP surfaces were obtained from PP black panels and had a semi-gloss finish with a thickness of 2 mm.	0/18 ICU "traps" 2/18 Covid wards "traps"	Not attempted.	Ct from positive surfaces were more than 10 cycles after than those obtained from the patient, indicating that the viral load was lower in the room environment.	NR	No-one could touch the surfaces of the 'traps" and patients were isolated in their rooms. RNA was found in two different surfaces at 72 h in the room of a patient with a nasal cannula. No positives were found at 24 or 48 h on the same surfaces. The rest of surfaces, placed in rooms with patients with no respiratory support, were not positive .
Razzini K 2020	Hospital	Italy	Observational; 5 air (& 37 surface) samples collected in the ICU for Covid-19 patients.	Air samples done using an MD8 Airport Portable Air Sampler with gelatine membrane filters, 1 filter for each monitored area. Each aspiration cycle was 40 min with a flow of 50 l/min. The detector was positioned 1.5 m above the floor. Air (n = 5) samples were collected from three zones classified as contaminated (corridor for patients and ICU), semi-contaminated (undressing room) and clean areas: (lockers and passage for the medical staff and a dressing room).	20/20 from the contaminated area 0/8 semi-contaminated 0/9 clean areas.	Not attempted.	Mean Ct for ICU air samples 22.6 Mean Ct for corridor air samples 31.1	NR	Surface contamination with SARS-CoV-2 RNA in Covid-19 wards was widespread, but not found in hospital "clean" areas. A total of 37 swab samples were collected within the five areas and 24% were positive for viral. Air samples collected from ICU and corridor for patients were positive for viral RNA with mean concentrations of 22.6 and 31.1 Ct value, respectively. In the present study the correlation between the virus concentration and the distance from patients was also evaluated. The Spearman coefficient suggests that there may be a moderate correlation and that the viral load of the surfaces increases with the patient proximity.

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Santarpia JL 2020a	Hospital	USA	Size-fractionated aerosol samples collected; virus culture was attempted.	Air samplers were placed in various places in the vicinity of the patient, including over 2m distant. Personal air sampling devices were worn by study personnel on two days during sampling. Measurements were made to characterize the size distribution of aerosol particles, and size-fractionated, aerosol samples were collected to assess the presence of infectious virus in particles sizes of >4.1 µm, 1-4 µm, and <1 µm in the patient environment. An Aerodynamic Particle Sizer Spectrometer was used to measure aerosol concentrations and size distributions from 0.542 µm up to 20 µm. A NIOSH BC251 sampler was used to provide size segregated aerosol samples for both rRT-PCR and culture analysis.	6/6 patient rooms.	In 3 aerosol samples of size <1 µm, cell culture resulted in increased viral RNA. Viral replication of aerosol was also observed in the 1 to 4 µm size but did not reach statistical significance.	Most RNA was identified in <1 microM particles (rather than 1-4 microM or >4.1 microM); concentrations up to around 7.5 TCID 50 / m3 of air.	Two of 1-4 µm samples demonstrated viral growth, between 90% and 95% confidence	Western blot assay was done using the antibody against SARS-CoV N protein, in cell supernatant samples with statistically significant evidence of replication. The presence of SARS-CoV-2 was reported to be observed via western blot for all but one of the samples (<1 um, Room 7B) with statistically significant evidence of replication, by rRT-PCR (Figure 2). Intact virus was reported to be observed via TEM in the submicron sample from Room 5 , and reported as "indicating active viral replication in that sample."
Santarpia JL 2020b	Healthcare centre	USA	High-volume (50 Lpm) and low-volume (4 Lpm) personal air samples (& surface samples) collected from 13 Covid-19 patients; virus culture was attempted.	We initiated an ongoing study of environmental contamination obtaining surface and air samples in 2 NBU hospital and 9 NQU residential isolation rooms housing individuals testing positive for SARS-CoV-2. Samples were obtained in the NQU on days 5–9 of occupancy and in the NBU on day 10	63% of in-room air samples positive (denominator unclear)	Cultivation of virus was not confirmed in these experiments. Authors suggest this was due to the low concentrations recovered in the samples.	Concentration of gene copies present in the recovered liquid sample (copies/µL): generally low and highly variable from sample to sample ranging from 0 to 1.75 copies/µL; with the highest concentration recovered from an air handling grate in the NBU.	NR	Aerosol samples were analyzed by RT-PCR targeting the E gene of SARS-CoV-2. Partial evidence of virus replication from one air sample. In the NBU, for the first two sampling events performed on Day 10, the sampler was placed on the window ledge away from the patient, and was positive for RNA (2.42 copies/L of air). On Day 18 in NBU Room B occupied by Patient 3, one sampler was placed near the patient and one was placed near the door greater than 2 meters from the patient's bed while the patient was receiving oxygen (1L) via nasal cannula. Both samples were positive by PCR, with the one closest to the patient indicating a higher airborne concentration of RNA (4.07 as compared to 2.48 copies/L of air). Samples taken outside the rooms in the hallways were 58% positive . Between 5 and 16 samples were collected from each room, with a mean of 7.35 samples per room and a mode of 6 samples per room.*in two of the samples, cell culture indicated some evidence for the presence of replication competent virus
Setti L 2020	Outdoor sampling	Italy	Observational study of particulate matter collected in industrial area of Bergamo over a continuous 3-week period	Particulate matter was collected using fiber filters by using a low-volume gravimetric air sampler (38.3 l/min for 24 h), compliant with the reference method EN12341:2014 for PM10 monitoring. This sampling procedure allows collection of aerosol and bioaerosol, by filtering 55 m3 per day, in a wide dimensional range; an approach considered suitable for sentinel and surveillance purposes.	20/34 PM samples positive for one gene 4/34 positive for 2 genes	Not attempted.	Not reported.	PM reported	particulate matter in defined conditions of atmospheric stability and high concentrations of PM10,
Seyyed Mahdi SM 2020	Hospital	Iran	Cross-sectional study in the Covid-19 ICU ward.	Air and surface sampling; impinger method was applied for air sampling: at a distance of 1.5 to 1.8 meters from the ground, the air of the ICU ward was passed through a sampling pump with an flow rate of 1.5 l/min into the porous midget impeller-30 ml containing 15 ml of virus transmission medium (PVTM) for 45 minutes.	6/10 air samples	Not attempted.	Highest RNA concentrations observed at the point between beds 6 and 7 (3913 copies per ml)	NR	Highest RNA concentrations observed at the point between beds 6 and 7 (3913 copies per ml). Most of the reported negative air samples were from the middle of the ward, which was further away from the patients beds. ten samples taken from different surfaces of the ward, 4 samples were positive (40%) and the highest concentration (8318 copies per ml) was related to the table next to bed number 3.

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Authors	Setting	Country	Method	Samples source	Air Samples PCR positive for SARs-CoV-2 RNA (unless otherwise stated)	Viral culture	Viral concentrations copies/m3 or copies/L or Cycle threshold (Ct)	size of air particles and proportion in sample	Notes
Shen Y 2020	Community including transport on buses	China	Observational epidemiology: cohort of 128 individuals.	128 individuals travelled on 1 of 2 buses to attend a worship event in Eastern China. Those who rode a bus with air recirculation and with a patient with COVID-19 had an increased risk of SARS-CoV-2 infection compared with those who rode a different bus.	Not attempted.	Not attempted.	N/A.	N/A.	Fomites and droplet transmission can not reasonably be excluded.
Song Z 2020	Public Health Clinical Center	China	Observational surveillance to evaluate the risk of viral transmission in AIIRs with 115 rooms in three buildings at the Shanghai Public Health Clinical Center, Shanghai, during the treatment of 334 patients infected with SARS-CoV-2.	In patient rooms, an air sampler was placed on the ground with a distance of about 1.0 m from patient's bed. In changing rooms, it was located between air supply outlet and air exhaust to capture particles from the unidirectional airflow. In addition, HEPA filters of air exhaust outlet in AIIRs in building 2 were collected.	0/7 ICU air samples 0/2 non ICU buildings.	Not attempted.	N/A (no air samples positive)	N/A.	We collected air samples from 15 AIIRs, including 7 ICU-AIIRs in building 3 and 8 non-ICU AIIRs in buildings 1 and 2. Two of the samples were collected in the ICU-AIIR of building 3 when tracheotomy surgery was performed. Directional airflow and strong environmental hygiene procedures were in place. None of 290 HCWs was infected when working in the AIIRs at this hospital. Additionally, testing of all surface samples from air exhaust and HEPA filters failed to detect any viral RNA
Tan L 2020	Hospital	China	Observational study of air and surface samples collected from isolation wards and ICU for 15 COVID-19 patients.	Air samples were obtained by placing an air sampler within 1 m of the patient's head; this continuously filtered air at a speed of 5 l/min and trapped small virus particles on a membrane. After 1 h the membrane was removed and cut into small pieces to be stored in VTM prior to further testing. The air sampler was placed at the same height as (or slightly lower than) an electronic fan installed on top of the windows to expel the air from the wards to the outside. Air samples were obtained from patient rooms, the corridor outside the patient rooms, and in the nearby nursing stations.	1/29 0/17 clean areas 1/12 patient rooms*	Not attempted.	Not attempted.	NR	*Only one sample was positive for SARS-CoV-2, which was collected within 10 cm of a female patient who was undergoing endotracheal intubation for invasive mechanical ventilation. SARS-CoV-2 RNA was not detected on any of the 36 surgical masks from 18 patients (14 mild and four severe/critical), although some of the patients had worn the same mask for 24 h. Most patients were 20 days post symptom onset, and 10/24 (42%) tested positive for SARS-CoV-2 by throat swab on the day of sampling.
Wei L 2020 (a)	Hospital	China	Sampled the surroundings and air of 6 negative-pressure non-ICU rooms	In a designated isolation ward occupied by 13 Covid-19 patients, including 2 asymptomatic patients. Air was sampled between 10:30 am and 13:00pm during the routine medical activities using an air sampler (FSC-1V; Hongrui, Suzhou, China) with 0.22-μm-pore-size filter membranes for 15 min at 100 liters/min. The air sampler was placed about 0.6 m away from each patient and 1 m above the floor in each room. The filter membranes were wiped by the use of pre moistened sterile swabs (Copan).	0/6 room air samples	Not attempted.	N/A (no air samples positive)	NR	3/6 of samples from air exhaust outlets in three rooms were positive for SARS-CoV-2. It appears that the patient surroundings in rooms with a SARS-CoV-2-positive air exhaust outlet are usually extensive contaminated (26.7% to 95.7%). It is possible that small virus-laden particles may be displaced by airflows and deposited on patient surroundings as suggested previously. 44/112 surface samples tested positive.

Authors	Setting	Country	Method	Samples source	Air Samples PCR positive for SARs-CoV-2 RNA (unless otherwise stated)	Viral culture	Viral concentrations copies/m3 or copies/L or Cycle threshold (Ct)	size of air particles and proportion in sample	Notes
Wei L (2020 (b)	Hospital	China	Observational study in patient surroundings and on PPE in a non-ICU isolation ward	The air from rooms for nine COVID-19 patients with illness or positive PCR >30 days, before and after nasopharyngeal/ oropharyngeal swabbing and before and after nebulization treatment. Air sampling was performed using an air microbiological sampler (FSC-1V; Hongrui, Suzhou, China) with 0.22 μm filter membranes on a nutrient agar plate for 15 min at 100 L/min, which was placed about 2 m away from patient and 1.1 m above the ground. Air was also sampled before and after performing nebulization treatment for all patients required (n= 4 on March 4 and n = 2 on March 12, 2020). After air sampling, the filters and the surface of agar were wiped using sterile swabs.	0/34 room air samples	Not attempted.	N/A (no air samples positive)	N/A	Environmental sampling was not performed at the early stage of COVID-19 when viral load in upper respiratory tract is higher. SARS-CoV-2 was not detected at distances > 1 m away from patients with a SARS-CoV-2-positive swab (n= 5 for nasopharyngeal and n= 2 for oropharyngeal) when collecting swabs nor after nebulization for patients with positive respiratory samples
Wong JCC 2020	Home residence	Singapore	Observational study of environmental contamination of SARS-CoV-2 in non 24 healthcare settings and assessed the efficacy of cleaning and disinfection in removing SARS-CoV-2 contamination.	Air samples were collected (n=4) in an accommodation room (occupied by Case 1) that was thought to be poorly ventilated and another 2 samples were collected right outside the room entrance. All samples were taken after the infected persons vacated the sites and have been isolated in healthcare facilities.	0/6 home residence samples	Not attempted.	N/A (no air samples positive)	N/A	Half of the surface swab and air samples were taken before the cleaning and disinfection and the other half was taken after the disinfection procedure, performed by third party commercial companies
Wong SCY 2020	Hospital	China	Case report and contact tracing and testing outbreak investigation of a patient in with COVID-19 who was nursed prior to Covid diagnosis in an open cubicle of a general hospital ward, Hong Kong.	Samples not collected.	Not attempted.	Not attempted.	N/A	N/A	Findings suggest that SARS-CoV-2 is not spread by an airborne route. A total of 71 staff and 49 patients were identified from contact tracing, 7 staff and 10 patients fulfilled the criteria of 'close contact'. After 28-day surveillance, 76 tests were performed on 52 contacts and all were negative, including all patient close contacts and 6/7 staff close contacts. The remaining contacts were asymptomatic throughout the surveillance period. Evidence against airborne transmission: no positive test among close contacts up to 28 days. The patient could not wear a surgical mask as she was on oxygen therapy through a simple facemask.
Wu S 2020	Hospital	China	Observational study of air and surface samples in hospital including rest rooms	Air samples from medical areas were collected through natural precipitation according to the Hygienic Standard for Disinfection in Hospitals.9 All samples were collected under emergency conditions around 8:00 AM before routine cleaning and disinfection	0/44 0/13 ICU 0/13 Wards 0/18 fever clinic	N/A	N/A	N/A	The positive rates in 200 environmental surface samples in medical areas (24.83%) was higher than that in living quarters (3.64%), with a significant difference (P < .05).

Authors	Setting	Country	Method	Samples source	Air Samples PCR positive for SARs-CoV-2 RNA (unless otherwise stated)	Viral culture	Viral concentrations copies/m3 or copies/L or Cycle threshold (Ct)	size of air particles and proportion in sample	Notes
Yuan XN 2020	Hospital	China	Observational study of the contaminated area in COVID-19 wards	Air samples from the clean area, the buffer room and the contaminated area in the COVID-19 wards using a portable bioaerosol concentrator WA-15.	0/90	Not attempted.	N/A (no air samples positive)	N/A	The 38 high-frequency contact surfaces samples of the contaminated area and 16 surface samples of medical staff's protective equipment including outermost gloves and isolation clothing were all negative.
Zhang D 2020	Outdoor environment of 3 hospitals	China	Air (and wastewater and soil samples) collected from the surroundings of a Covid-19 hospital.	73 air and wastewater samples from the environment of three hospitals in Wuhan treating Covid-19 patients.	3/16	Not attempted.	SARS-CoV-2 RNA found in aerosols ranged from 285 to 1,130 copies/m3. Inside the adjusting tank of Hospital 1 and Hospital 2, respectively, SARS-CoV-2 in aerosols was found at a level of 285 copies/m3 and 603 copies/m3. Aerosols collected 5 m away from Hospital 1 outpatient building were 1130 copies/m3, whereas undetected in aerosols collected 5 m away from the inpatient building.	N/A	SARS-CoV-2 RNA found in a range of samples adjacent to hospitals. Inside the adjusting tank of Jinyintan Hospital and Huoshenshan Hospital, SARS-CoV-2 in aerosols was found at a level of 285 copies/m3 and 603 copies/m3. Outside patient departments of Jinyintan Hospital, SARS-CoV-2 in the aerosols collected 5 m away from outpatient building were 1130 copies/m3, whereas undetected in aerosols collected 5 m away inpatient building.
Zhou J 2020	Hospital	UK	Observational: (air & surface) samples collected from a hospital with a high number of Covid-19 inpatients.	In the Emergency Department dedicated for patients with confirmed or suspected COVID-19, two of the cubicles were occupied and one patient was in the ambulatory wait area at the time of sampling. These areas were disinfected daily using a combined chlorine-based detergent/disinfectant (Actichlor Plus, Ecolab), with an additional twice daily disinfection of high touch surfaces using the same detergent/disinfectant. In each of these clinical areas, four air samples were collected (five air samples were collected in the Emergency Department, and three in public areas of the hospital). Air sampling was performed using a Coriolis μ air sampler (referred to as Coriolis hereafter) (Bertin Technologies), which collects air at 100–300 litres per minute (LPM). After 10 min sampling at 100 LPM, a total of 1.0 m3 147 air was sampled into a conical vial containing 5 mL Dulbecco's minimal essential medium (DMEM).	2/31 air samples positive 12/31 suspected	0/14	101 to 103 copies of SARS-CoV-2 RNA was detected in all air samples; no significant difference between sample areas.	NR	We defined samples where both of the PCRs performed from an air or surface sample detected SARS-CoV-2 RNA as positive, and samples where one of the two PCRs performed from an air or surface sample detected SARS-CoV-2 RNA as suspected
Zhou L 2020	Hospital	China	Study of collected samples of exhaled breath of patients ready for discharge and air samples.	The 13 patients in 4 hospitals were aged 70+ years. 10 were recovered Covid-19 patients ready for discharge; 3 were patients recovered from influenza who tested negative for SARS-CoV-2). Air (& surface) samples were collected. Exhaled breath condensate of 300-500 L was collected from each patient: a long straw was used to allow the patient to breathe into a tube that was electrically cooled. 44 air samples were taken, from corridors, hospital waste storage rooms, ICU rooms (5 samples), toilets, medical preparation rooms, clinical observation rooms, and general wards. Two impinger samplers were used: WA-15 sampled at a flow rate of 15 L/min, while the WA-400 sampled at 400 L/min.	0/44	Not attempted.	N/A (no air samples positive)	Not attempted.	1.3% of surface swab samples tested positive.20% of Covid-19 patients, who were ready for a hospital discharge based on current guidelines, had SARS-CoV-2 in their exhaled breath (~105 RNA copies/m3); They were estimated to emit about 1400 RNA copies into the air per minute.

Table 2. Study characteristics: reviews.

Study (n=22)	Fulfils systematic review methods	Research question (search date up to)	No. included studies (No. participants)	Main results	Key conclusions
Airborne transmission and its prevention (n=14)					
Anderson EL 2020	no	What are the scientific uncertainties and potential importance of aerosol transmission of SARS-CoV-2. (**search methods and date not clear**)	unclear	Limited evidence reports that SARS-CoV-2 can remain active in aerosol for at least 3 hours, although its concentration decreases over time.	Further data collection required assessment under differing conditions of temperature and humidity. Such research should be relatively low cost and results available in a short time.
Agarwal 2020	yes	To summarize the evidence for the efficacy, safety, and risk of aerosol generation and infection transmission during high-flow nasal cannula (HFNC) use among patients with acute hypoxemic respiratory failure due to COVID-19 (**search conducted to 14 May**)	Four studies evaluating droplet dispersion and three evaluating aerosol generation and dispersion.	Two simulation studies and a crossover study showed mixed findings regarding the effect of HFNC on droplet dispersion. Two simulation studies reported no associated increase in aerosol dispersion, and one reported higher flow rates were associated with increased regions of aerosol density (evidence rated as very low certainty).	High-flow nasal cannula may reduce the need for invasive ventilation and escalation of therapy
Bahl P 2020	no	We aimed to review the evidence supporting the rule of 1-meter (≈3 feet) spatial separation for droplet precautions in the context of guidelines issued by the WHO, CDC, and European Centre for Disease Prevention and Control (ECDC) for HCWs on respiratory protection for COVID-19. (**open search to March 2020**)	Ten papers were included in the review	We found that the evidence base for current guidelines is sparse, and the available data do not support the 1- to 2-meter (≈3–6 feet) rule of spatial separation. Of 10 studies on horizontal droplet distance, 8 showed droplets travel more than 2 meters (≈6 feet), in some cases up to 8 meters (≈26 feet). Several studies of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) support aerosol transmission, and 1 study documented virus at a distance of 4 meters (≈13 feet) from the patient.	The weight of combined evidence supports airborne precautions for the occupational health and safety of health workers treating patients with COVID-19.
Birgand G 2020 and Birgand G 2020 JAMA	no	Evidence for airborne contamination of SARS-CoV-2 in hospitals (**search conducted to 21 Jul**y repeated on October 27, 2020 for JAMA publication)	17 articles JAMA Dec: 24 cross-sectional observational studies	68/247 (28%) of air sampled from close patients environment were positive for SARS-CoV-2: no difference according to the setting (ICU: 27/97, 27.8%; non-ICU: 41/150, 27.3%; p=0.93), or the distance from patients (<1 metre: 1/64, 1.5%; 1 to 5 metres: 4/67, 6%; p=0.4). 3/78 (4%) viral cultures performed in three studies were positive (all were samples from close to patients). JAMA: A total of 81 viral cultures were performed across 5 studies, and 7 (8.6%) from 2 studies were positive, all from close patient environments.	In hospital, the air near and away from COVID-19 patients is frequently contaminated with SARS CoV-2 RNA, with however, rare proofs of their viability. **JAMA** in this systematic review, the air close to and distant from patients with coronavirus disease 2019 was frequently contaminated with SARS-CoV-2 RNA; however, few of these samples contained viable viruses. High viral loads found in toilets and bathrooms, staff areas, and public hallways suggest that these areas should be carefully considered.
Carducci A 2020	no	To describe the state of the art of coronavirus airborne transmission (**search conducted 5 June**)	64 papers classified into three groups: laboratory experiments (12 papers), air monitoring (22) and epidemiological and airflow model studies (30	Airborne transmission of SARS-CoV-2 was suggested by studies across the three groups, but methods were not standardised.	No studies had sufficient confirmatory evidence, and there is only a hypothesis to support airborne transmission
Chen PZ 2020	yes	To develop a comprehensive dataset of respiratory viral loads (rVLs) of SARS-CoV-2, SARS-CoV-1 and influenza A(H1N1)pdm09 (**search conducted to 7 Aug**)	64 studies (n = 9,631 total specimens)	Modelling of the likelihood of respiratory particles containing viable SARS-CoV-2. When expelled by the mean COVID-19 case during the infectious period, respiratory particles showed low likelihoods of carrying viable SARS-CoV-2. Aerosols (equilibrium aerodynamic diameter [da] ≤ 100 μm) were ≤0.69% (95% CI: 0.43-0.95%) likely to contain a virion. Droplets also had low likelihoods: at a equilibrium aerodynamic diameter = 330 μm,	Aerosols (≤100 μm) can be inhaled nasally, whereas droplets (>100 μm) tend to be excluded. For direct transmission, droplets must be sprayed ballistically onto susceptible tissue. Hence, droplets predominantly deposit on nearby surfaces, potentiating indirect transmission. Aerosols can be further categorized based on typical travel characteristics: short-range aerosols (50–100 μm) tend to settle within 2 m; long range ones (10–50 μm) often travel beyond 2 m based on emission force; and buoyant aerosols (≤10 μm) remain suspended and travel based on airflow profiles for minutes to many hours

Study (n=22)	Fulfils systematic review methods	Research question (search date up to)	No. included studies (No. participants)	Main results	Key conclusions
Comber L 2020	yes	To synthesise the evidence for the potential airborne transmission of SARS-CoV-2 via aerosols. (**Searches 1 Jan up to 27 July 2020)**.	28 studies (8 epidemiological case series of SARS-CoV-2 clusters or outbreaks;16 air sampling studies, and 4 virological studies).	10/16 air sampling studies detected SARS-CoV-2 ribonucleic acid; however, only three of these studies attempted to culture the virus with one being successful in a limited number of samples. Two of four virological studies using artificially generated aerosols indicated that SARS-CoV-2 is viable in aerosols.	The results of this review indicate there is inconclusive evidence regarding the viability and infectivity of SARS-CoV-2 in aerosols. Epidemiological studies suggest possible transmission, with contextual factors noted. However, there is uncertainty as to the nature and impact of aerosol transmission of SARS-CoV-2, and its relative contribution to the Covid-19 pandemic compared with other modes of transmission.
Ekram W 2020	no	To summarize the ways in which SARS-CoV-2 is transmitted **(Searches Dec 28, 2019 up to July 31 2020)**	unclear	Evidence-based hypotheses support the possibility of SARS-CoV-2 airborne transmission due to its persistence in aerosol droplets in a viable and infectious forms.	Aerosolized transmission is likely the dominant route for the spread of SARS-CoV-2, particularly in healthcare facilities. Although SARS-CoV-2 has been detected in non-respiratory specimens, including stool, blood and breast milk, their role in transmission remains uncertain.
Ji B 2020	no	To reviews the information from published papers, newsletters and large number of scientific websites to profile the transmission characteristics of the coronaviruses in water, sludge, and air environment, (**search methods and date not clear)**	unclear		It appears that the wastewater, sludge, aerosol are potentially environmental transmission of coronavirus.
Mehraeen E 2020	no	To review the current evidence of COVID-19 transmission modes. **(Searches Dec 2019 to April 2020)**	36 studies including 31 articles (11 reports, eight reviews, seven letters to the editor, two modeling, one perspective, and two experimental studies) and five clinical trials.	Identified five potential transmission modes of COVID-19 including airborne, droplet, contact with contaminated surfaces, oral and fecal secretions.	Droplet and contact with contaminated surfaces were the most frequent transmission modes of COVID-19. Fecal excretion, environmental contamination, and fluid pollution might contribute to a viral transmission
Niazi S 2020	no	To evaluate the mechanisms of generation of human pathogenic coronaviruses, evaluating these viruses in the air/field studies and available evidence about their seasonality patterns. (**searches no restriction on year up to July 31 2020)**	total unclear (8 Studies of air sampling: 6 Sars-CoV-2)	Evidence exists for respirable-sized airborne droplet nuclei containing viral RNA, although this does not necessarily imply that the virus is transmittable, capable of replicating in a recipient host, or that inoculum is sufficient to initiate infection. However, evidence suggests that coronaviruses can survive in simulated droplet nuclei for a significant time (>24 h). Nevertheless, laboratory nebulized virus-laden aerosols might not accurately model the complexity of human carrier aerosols in studying airborne viral transport	Human respiratory activities generate respirable sized aerosols that are of adequate size to support an infectious virus. Knowledge of the properties of respiratory aerosols and their effects on the viability of viruses remains incomplete. Environmental factors could directly affect the viability of virus on the embedded viruses in aerosols. There is disagreement on whether wild coronaviruses can be transmitted via an airborne path. Further studies are required to provide supporting evidence for the role of airborne transmission.
Noorimotlagh Z 2020	no	to review studies on airborne transmission of SARS-CoV-2 in indoor air environments.(**search methods and date not clear)**	14 studies	11 studies were experimental and reported different findings on positive or negative detection of SARS-CoV-2 airborne transmission in indoor air. Among them, three studies indicated that all indoor air samples in the hospital were negative, thus concluding that there is no evidence that SARS-CoV-2 is transmitted by air (Faridi *et al.*, 2020; Kim *et al.*, 2020; Masoumbeigi *et al.*, 2020). the other included experimental studies reported positive results that confirmed transmission of the virus through the air.	There is a possibility of airborne transmission of SARS-CoV-2 in indoor air environments.
Rahmani 2020	no	A review of methods used for sampling and detection of SARS like viruses in the air. (**search methods and date not clear)**	not clear	Factors that limit the interpretation included variable patient distance from the sampler, use of protective or oxygen masks by patients, patient activities, coughing and sneezing during sampling time, air movement, air conditioning, sampler type, sampling conditions, storage and transferring conditions.	Most studies are not able to discriminate between airborne or respiratory droplet transmission.

Study (n=22)	Fulfils systematic review methods	Research question (search date up to)	No. included studies (No. participants)	Main results	Key conclusions
Ren SY 2020	No	This review aims to summarize data on the persistence of different coronaviruses on inanimate surfaces. **(search date unclear)**	unclear	Viruses in respiratory or fecal specimens can maintain infectivity for quite a long time at room temperature. Absorbent materials like cotton are safer than unabsorbent materials for protection from virus infection. The risk of transmission via touching contaminated paper is low. Preventive strategies such as washing hands and wearing masks are critical to the control of coronavirus disease 2019.	Viruses in respiratory or fecal specimens can maintain infectivity for quite a long time at room temperature. Absorbent materials like cotton are safer than unabsorbent materials for protection from virus infection. The risk of transmission via touching contaminated paper is low.
Singhal S 2020	no	To focus on different modes of transmission of this virus, comparison of this virus with previous similar analogy viral diseases like SARS and MERS (**Searches Jan 1 to 29 April 2020)**	unclear		
Wilson NM 2020	no	To assess the airborne transmission of severe acute respiratory syndrome coronavirus-2 to healthcare workers (**search methods and date not clear)**	unclear	Evidence largely from low-quality case and cohort studies where the exact mode of transmission is unknown as aerosol production was never quantified. The mechanisms and risk factors for transmission were also largely unconfirmed.	Limited evidence suggests aerosol generating procedures cause an increase in airborne healthcare worker transmission. Further research is required.
Airborne transmission and procedures (n=3)					
Hussain A 2020	no	Extent of infectious SARS-CoV-2 aerosolisation as a result of oesophagogastroduodenoscopy or colonoscopy **(search conducted up to 5 June)**	26 studies	The aerosolisation and infectious extent of SARS-CoV-2 cannot be accurately measured, and no clinical studies have confirmed aerosol infection of SARS-CoV-2,	
Kay JK 2020	yes	What is the evidence for minimizing the use of flexible laryngoscopy during the coronavirus disease 2019 pandemic? **(search conducted upto April 2020)**	No studies provided data for SARS-CoV-2 transmission during flexible laryngoscopy.	A paucity of data regarding the risks of SARs-CoV-2 aerosolization and transmission during endoscopic procedures of the aerodigestive tract	More research is needed.
Schünemann HJ	yes	To review multiple streams of evidence regarding the benefits and harms of ventilation techniques for coronavirus infections, including that causing COVID-19 (**search conducted up to 1 May).**	123 (45 on COVID-19)	Evidence suggests an increased risk for transmission of coronaviruses with invasive procedures. An additional 34 studies in COVID-19 patients were found, by their methods and reporting were too poor to synthesize data appropriately.	Direct studies in COVID-19 are limited and poorly reported.
Ventilation, air conditioning filtration and recirculation (n=3)					
Mousavi EH 2020	no	What is the safety of air filtration and air recirculation in healthcare premises. **(search methods and date not clear**)	109 documents categorized into five levels	Evidence to support current practice is very scarce. No randomized trials were retrieved and most experiments were designed to try to prove airborne transmission as opposed to test the null hypothesis. Observational evidence and animal studies showed contaminated air can result in disease spread, and the combination of air filtration and recirculation can reduce this risk.	There is a need for a rigorous and feasible line of research in the area of air filtration and recirculation in healthcare facilities.
Chirico F 2020	no	What is the impact of heating, ventilation and air conditioning systems (HVAC) on transmission of coronaviruses (**search conducted 11 July)**	Six studies on SARS-CoV-2	In three of six studies of SARS-CoV-2, the heating and ventilation system was suspected to aid transmission; in two studies the data did not support such an effect, and in one study only modelling suggested an impact	The differences in HVAC systems prevent generalization of the results. The few investigations available do not provide sufficient evidence that SARS-CoV-2 can be transmitted by HVAC systems.
Correia G 2020	no	What is the impact of HVAC in hospitals or healthcare facilities on the spread of the virus. **(search methods and date not clear)**	unclear		The authors speculate that incorrect use of HVACs might contribute to the transmission of the virus.

Table 3. Quality of included studies.

Study	Is the source popn adequately described	Description of methods and sufficient detail to replicate	Samples sources clear and quantified	Analysis & reporting outcomes appropriate	Was follow up sufficient
Ahn JY 2020	Yes	Yes	No	Unclear	Not Applicable
Bays D 2020	Yes	Yes	Not Applicable	Yes	Yes
Binder 2020	Yes	Yes	Yes	Yes	Yes
Charlotte N 2020	Yes	Unclear	Not Applicable	Unclear	Yes
Cheng VCC 2020a	Yes	Yes	Yes	Yes	Not Applicable
Cheng VCC 2020b	Unclear	Yes	Yes	Unclear	Not Applicable
Chia PY 2020	Yes	Yes	Yes	Yes	Not Applicable
Chirizzi D 2020	Not Applicable	Yes	Yes	Yes	Not Applicable
Declementi M 2020	Yes	Yes	Yes	Yes	Not Applicable
De Man P 2020	Unclear	Yes	Not Applicable	Unclear	Not Applicable
Di Carlo P 2020	Not Applicable	Yes	Yes	Yes	Not Applicable
Ding Z 2020	Yes	Yes	Yes	Unclear	Not Applicable
Döhla M 2020	Unclear	Yes	Yes	Unclear	Not Applicable
Dumont-Leblond 2020	Yes	Yes	Yes	Yes	Not Applicable
Faridi S 2020	Yes	Yes	Yes	Yes	Not Applicable
Feng B 2021	Yes	Yes	Yes	Yes	Not Applicable
Ge XY 2020	Yes	Unclear	Yes	Unclear	Not Applicable
Günther T 2020	Yes	Yes	Yes	Unclear	Yes
Guo ZD 2020	Yes	Yes	Yes	Yes	Not Applicable
Hamner 2020 and Miller SL 2020	Yes	Yes	Not Applicable	Unclear	Yes
Hernández JL 2020	Unclear	Yes	Yes	Yes	Not Applicable
Horve PF 2020	Yes	Yes	Yes	Yes	Not Applicable
Hu J 2020	Yes	Yes	Yes	Unclear	Not Applicable
Jiang Y 2020	Yes	Yes	Unclear	Unclear	Not Applicable
Jin T 2020	Yes	Yes	Yes	Yes	Not Applicable
Kang M 2020	Yes	Yes	Unclear	Unclear	Not Applicable
Kenarkoohi A 2020	Yes	Yes	Yes	Unclear	Not Applicable
Kim UJ 2020	Yes	Yes	Yes	Yes	Not Applicable
Kwon KS 2020	Yes	Yes	Not Applicable	Yes	Yes
Lednicky JA 2020a	Yes	Yes	Yes	Unclear	Not Applicable
Lednicky JA 2020b	Yes	Yes	Yes	Unclear	Not Applicable
Lei H 2020	Yes	Yes	Yes	Yes	Not Applicable
Li Y & Qian H 2020	Yes	Yes	Not Applicable	Yes	Yes
Li YH & Fan YZ 2020	Yes	Yes	Yes	Yes	Not Applicable
Lin G 2020	Yes	Yes	Not Applicable	Yes	Not Applicable
Liu Y, Ning Z 2020	Yes	Yes	Yes	Yes	Not Applicable

Study	Is the source popn adequately described	Description of methods and sufficient detail to replicate	Samples sources clear and quantified	Analysis & reporting outcomes appropriate	Was follow up sufficient
Lu J 2020	Yes	Unclear	Not Applicable	Unclear	Not Applicable
Luo K 2020	Yes	Yes	Not Applicable	Yes	Yes
Ma J 2020	Yes	Yes	Yes	Yes	Not Applicable
Marchetti 2020	Yes	Yes	Unclear	Unclear	Not Applicable
Masoumbeigi 2020	Yes	Yes	Yes	Yes	Not Applicable
McGain F	Yes	Yes	Unclear	Unclear	Not Applicable
Moreno 2020	Not Applicable	Yes	Yes	Yes	Not Applicable
Morioka S 2020	Yes	Yes	Not Applicable	Unclear	Not Applicable
Mponponsuo K 2020	Yes	Yes	Not Applicable	Yes	Yes
Nakamura K 2020	Yes	Yes	Yes	Yes	Not Applicable
Nissen K 2020	Yes	Unclear	Yes	Unclear	Not Applicable
Ogawa Y 2020	Yes	Yes	Yes	Yes	Yes
Ong SWX 2020	Yes	Yes	Yes	Yes	Not Applicable
Orenes-Piñero E 2020	Yes	Yes	Not Applicable	Yes	Not Applicable
Razzini K 2020	Yes	Yes	Yes	Yes	Not Applicable
Santarpia JL 2020a	Yes	Yes	Yes	Unclear	Not Applicable
Santarpia JL 2020b	Yes	Yes	Yes	No	Not Applicable
Setti L 2020	Not Applicable	Yes	Yes	Yes	Not Applicable
Seyyed Mahdi SM 2020	Yes	Yes	Yes	Unclear	Not Applicable
Shen Y 2020	Unclear	Yes	Not Applicable	No	Unclear
Song Z 2020	Unclear	Yes	Yes	Yes	Not Applicable
Tan L 2020	Yes	Yes	Yes	Unclear	Not Applicable
Wei L 2020a	Yes	Yes	Yes	Yes	Not Applicable
Wei L 2020b	Yes	Yes	Yes	Yes	Not Applicable
Wong JCC 2020	Yes	Yes	Unclear	Yes	Not Applicable
Wong SCY 2020	Yes	Yes	Not Applicable	Yes	Yes
Wu S 2020	Yes	Unclear	Yes	Unclear	Not Applicable
Yuan XN 2020	Unclear	Unclear	Unclear	Unclear	Not Applicable
Zhang D 2020	Yes	Unclear	Yes	Unclear	Not Applicable
Zhou J 2020	Yes	Yes	Yes	Yes	Not Applicable
Zhou L 2020	Yes	Yes	Yes	Yes	Not Applicable
Total	56	60	46	39	11
	67	67	67	67	67
	83.6%	89.6%	68.7%	58.2%	16.4%

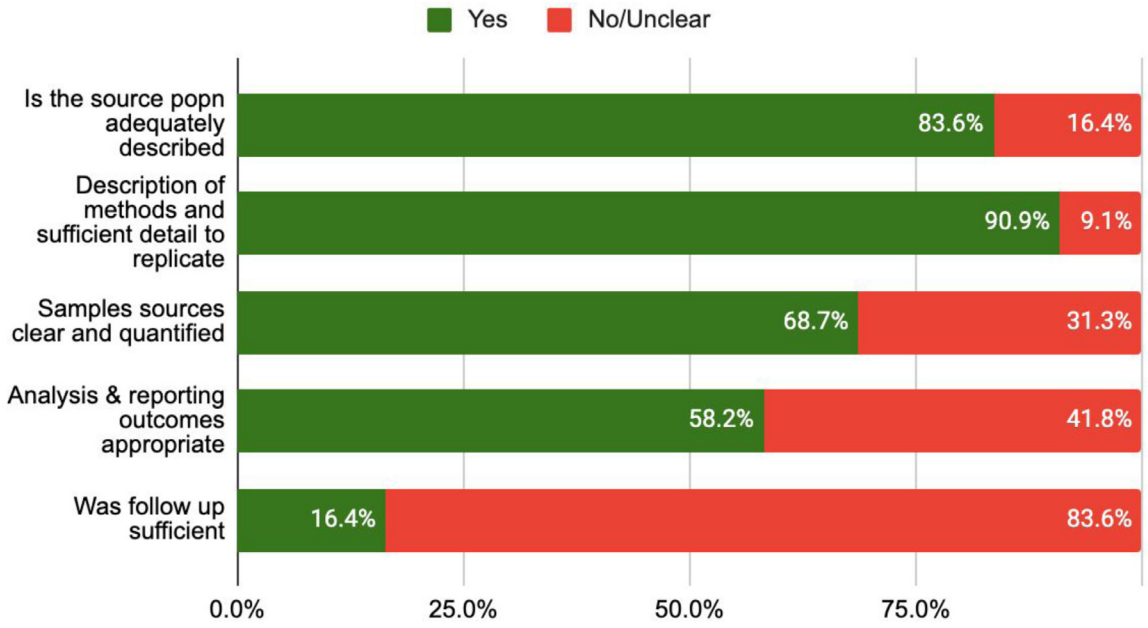

Figure 2. **Risk of bias (n=67).**

Table 4. **Concentrations of PCR samples recovered (n=25).**

Study (n=64)	Cycle Threshold (Ct)	Copies per m^3 (or L)
Binder 2020	Sample at 1.4m, <4uM: 1st 36.6; 2nd 37.1 Sample at 2.2m, <4uM: 1st 37.4, 2nd 39.9 Sample at 2.2m, >4uM: 1st 39.1, 2nd 39.6	
Chia PY 2020		range 1.84×10^3 to 3.38×10^3 RNA copies per m^3
Chirizzi D 2020		<0.8 copies m^3 for each size range.
Ding Z 2020		RNA copies for weakly positive sample not calculated.
Dumont-Leblond N 2020	N gene (range 36.5 to 39.8) mean 38.0 ORF1b gene (32.1 to 35.2) mean 33.7	8 positives for both N and Orf1b (range 9.9 to 514.2) mean 201.6 genomes /m^3
Feng B 2020		<1 µm: 1,111 copies/m^3 >4 µm: 744 copies/m^3
Ge XY 2020	ICU: Ct 36.5 - 37.8	
Guo ZD 2020	Indoor air near air outlet: Ct 35.7, Near patients: Ct 44.4. Near the doctor's office area: Ct 12.5	Indoor air near the air outlet: 3.8/L near the patients: 1.4/L near the doctor's office area: 0.52
Horve PF 2020		The highest abundance sample (~245 gene copies) found on the pre-filters,
Hu J 2020		range 1.11×10^3 to 1.12×104 copies m^3 In 10% of outdoor air samples, 10 m from the doors of inpatient & outpatient buildings range 0.89 to 1.65×10^3 copies m^3
Kenarkoohi A 2020	Ct around 38 for ORF1ab Ct around 35 for n gene	

Study (n=64)	Cycle Threshold (Ct)	Copies per m^3 (or L)
Lednicky JA 2020a	Ct 36.0, 37.7, 37.4, 38.7, respectively (mean Cq 37.5)	Four positives contain: 2.82E+03, 9.12E+02, 1.15E+03, 4.68E+02 genome equivalents/25 µL,
Lednicky JA 2020b	Ct 39.1	0.87 virus genome equivalents L^{-1}
Lei H 2020	Near the head of the patient Ct 41.25.	
Liu Y & Ning Z 2020		ICU: rang- 0 -113 copies m^3 Patient areas 0 -19 copies m^3 Medical Staff Areas 0 - 42m^3 Public areas: 0 -11copies m^3
Ma J 2020	Exhaled Breath Samples, 14 positives: Ct 35.5 ± 3.2	Breath emission rate estimate: 1.03 × 10^5 to 2.25 × 10^7 viruses per hour. Air sample estimate 6.1 × 10 3 viruses/m^3
Moreno T 2020		genome count range 14 to 446/m2 for IP2, 9 to 490/m2 for IP4 and 5 to 378/m2 for E. Subway: 1st sample estimate 23.4 GC/m^3, 2nd amplified target gene IP2 (18.8 GC/m^3) & protein E (5.6 GC/m^3).
Nissen K 2020	Ct N gene: 35.3 Ct E gene 33.2 Ward 1 specimen Ct 33.0 for E gene only.	
Orenes-Piñero E 2020	Ct from surfaces > 10 cycles of those obtained from the patient, indicating viral load was lower in the room environment.	
Razzini K 2020	ICU: Mean Ct 22.6 Corridor: Mean Ct 31.1	
Santarpia JL 2020a		concentrations up to around 7.5 TCID 50 / m3 of air.
Santarpia JL 2020b		gene copies generally low and highly variable from sample to sample ranging from 0 to 1.75 copies/µL
Seyyed Mahdi SM 2020		Highest RNA concentrations observed between beds 6 and 7 (3,913 copies per ml)
Zhang D 2020		Range 285 to 1,130 copies/m3. Inside adjusting tank 285 copies/m3 and 603 copies/m^3. 5 m from Hospital outpatient building 1,130 copies/m^3, 5 m from the inpatient building undetected
Zhou J 2020		101 to 103 copies of SARS-CoV-2 RNA in all air samples; no significant difference between sample areas.

Two studies conducted in hospitals also sampled other spaces. Liu Y & Ning Z *et al.* reported 4/13 public areas were RT-PCR positive; Ma J *et al.* reported 1 positive sample from an unventilated quarantine hotel toilet room out of 26 samples taken. Zhang D *et al.* sampled the outdoor environment of three hospitals and reported 3/16 samples were RT-PCR positive. Lednicky JA 2020b sampled in a respiratory infection evaluation area of a student health care center and reported one positive sample with a CT of 39 (virus genome equivalent of 0.87 virus genomes L^{-1} air).

Two studies reported on Exhaled Breath Condensate (EBC). Ma J *et al.* reported 14/52 EBC samples as RT-PCR positive and Feng B *et al.* reported 2/8 positive EBC samples. Five studies conducted in hospitals did not attempt RT-PCR air sampling [Bays D 2020; McGain F 2020; Mponponsuo K 2020 Ogawa Y 2020 and Wong SCY 2020] In Lei H *et al.*, it was not possible to separate air from surface sample results.

Outdoors and community. Seventeen studies reported on the outdoors and in the community (see Figure 1). These settings were buses (four studies: two in china [Luo K 2020 and Shen Y 2020]; one in Italy [Di Carlo P 2020] and one from Spain that included subway trains [Moreno T 2020]); two studies each for the outdoors; restaurant; choir practice & block of flats, and one study each for a meat processing plant; home

Table 5. The size of air particles in the sample (n=8).

Study (n=64)	Samples Source	Size of air particles
Binder 2020	8 National Institute for Occupational Safety and Health (NIOSH) BC 251 Aerosol Samplers (Figure S3) were placed 1.5m from the ground, at ~1 meter, ~1.4 meters, ~2.2 meters, and ~3.2 meters from the SARS-CoV-2 patient's head and subsequently run for ~4 hours. 195 air samples were collected	detected in aerosols particle size <4 μm
Chia PY 2020	Air sampling was performed in three of the 27 airborne infection isolation rooms (AIIRs). Bioaerosol samplers used to collect air samples, set at a flow-rate of 3.5 L/min and run for four hours, collecting a total of 5,040 L of air from each patient's room.	positive particles of sizes >4 μm and 1–4 μm detected in two rooms
Chirizzi D 2020	The genetic material of SARS-CoV-2 (RNA) was determined, using both real-time RT-PCR and ddPCR, in air samples collected using PM10 samplers and cascade impactors able to separate 12 size ranges from nanoparticles (diameter D < 0.056 μm) up to coarse particles (D > 18 μm).	(D < 0.056 μm) up to coarse particles (D > 18 μm)
Feng B 2020	For a sampling of isolation room air, a NIOSH sampler was placed on a tripod 1.2 m in height and 0.2 m away from the bed at the side of the patient's head. The sampling duration was 30 min, and a total of 105-L room air was sampled. (9 Exhaled Breath (EB) samples, 8 Exhaled Breath Condensate (EBC) samples, 12 bedside air samples)	RNA detected in the air sample in <1 μm and >4 μm fractions,
Hernández JL 2020	Air sampled in three areas: Emergency area (Clinic A), Internal medicine (Clinic A), COVID 19 patient area (Clinic A), and COVID-19 patients care room (Clinic B). Sampling in all areas was accomplished in 3 h. Filters of 25 mm diameter with 0.22 μm pores were utilized (Millipore, AAWP02500), placed in a sterilized filter holder (Millipore, SWINNX) coupled to a vacuum system through a previously disinfected plastic hose, filtering the air with a flow of 9.6 L/min in each filter holder.	filtration through 0.22 μm pores.
Liu Y & Ning Z 2020	Over a 2 week period: 30 aerosol samples of total suspended particles collected on 25-mm-diameter filters loaded into styrene filter cassettes (SKC) by sampling air at a fixed flow rate of 5.0 l min−1 using a portable pump (APEX2, Casella). Three size-segregated aerosol samples collected using a miniature cascade impactor (Sioutas Impactor, SKC) that separated aerosols into five ranges (>2.5 μm, 1.0 to 2.5 μm, 0.50 to 1.0 μm and 0.25 to 0.50 μm on 25-mm filter substrates, and 0 to 0.25 μm on 37-mm filters) at a flow rate of 9.0 l min−1. Two aerosol deposition samples collected using 80-mm-diameter filters packed into a holder with an effective deposition area of 43.0 cm2; filters were placed intact on the floor in two corners of an ICU for 7 days.	SARS-CoV-2 aerosols one in the submicrometre region (dp between 0.25 and 1.0 μm) and the other in supermicrometre region (dp > 2.5 μm). Aerosols in the submicrometre region were found with peak concentrations of 40 and 9 copies m3 in the 0.25–0.5 μm and 0.5–1.0 μm range, respectively.
McGain F 2020	Two spectrometers to measure aerosol particles: the portable Mini Wide Range Aerosol Sizer 1371 (MiniWRAS) and the Aerodynamic Particle Sizer (APS). During the procedure, the aerosol detector inlet was positioned 30 cm directly above the patient's neck, representing the surgeon's breathing air space	APS detected larger aerosols (> 0.37 mm) and MiniWRAS smaller particles (0.01–0.35 mm).

Study (n=64)	Samples Source	Size of air particles
Santarpia JL 2020a	Air samplers were placed in various places in the vicinity of the patient, including over 2m distant. Personal air sampling devices were worn by study personnel on two days during sampling. Measurements were made to characterize the size distribution of aerosol particles, and size-fractionated, aerosol samples were collected to assess the presence of infectious virus in particles sizes of >4.1 µm, 1-4 µm, and <1 µm in the patient environment. An Aerodynamic Particle Sizer Spectrometer was used to measure aerosol concentrations and size distributions from 0.542 µm up to 20 µm. A NIOSH BC251 sampler18 was used to provide size segregated aerosol samples for both rRT-PCR and culture analysis.	Two of the 1-4 µm samples demonstrated viral growth, between 90% and 95% confidence

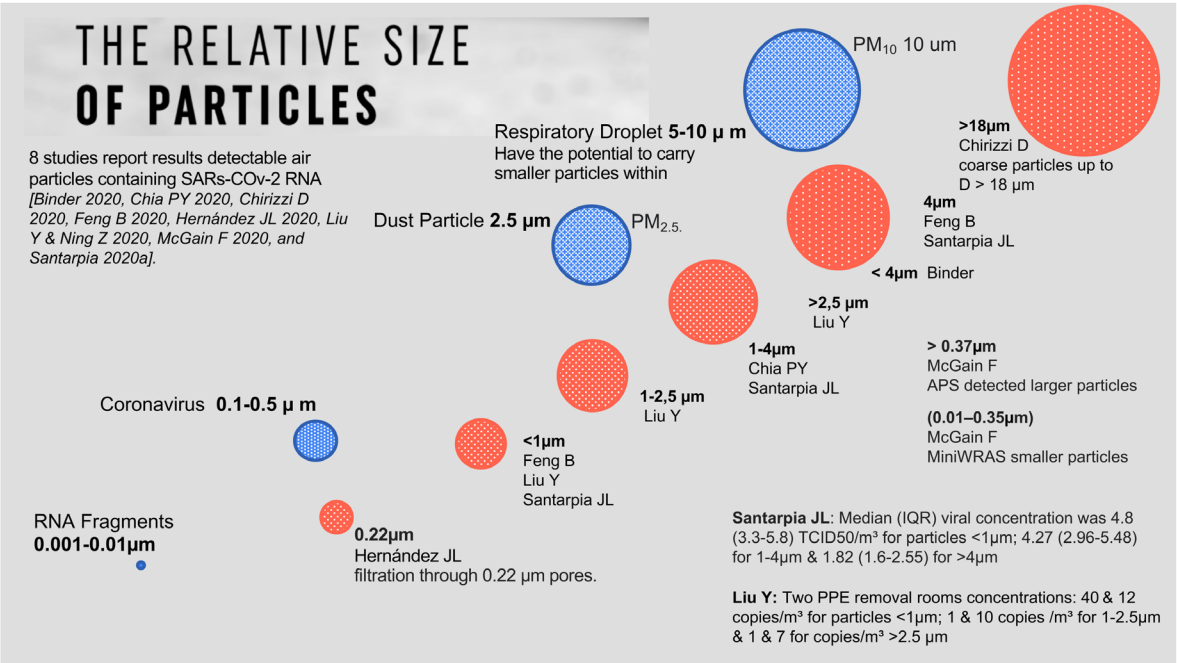

Figure 3. The relative size of particles.

residence; quarantine hotel; quarantined household and a care home.

Seven of these studies undertook RT-PCR sampling [Di Carlo P 2020: inside a bus; Dohla M 2020: quarantined households; Kang M 2020: a block of flats; Kwon KS 2020: the community; Moreno T 2020; buses and subway trains; Setti L 2020: outdoor sampling; and Wong JCC 2020: in the home residence], and one Chirizzi D 2020 sampled atmospheric concentrations.

Of the eight studies, two reported positive RT-PCR samples (5 of 125 samples positive for 2 or more genes, average 4.0%), and one Chirizzi 2020 *et al.* found outdoor atmospheric concentrations of SARS-CoV-2 RNA at low levels <0.8 copies m^3. Moreno 2020 *et al.* sampled on buses and subway trains in Barcelona, and reported samples were mainly positivity for only 1 of the 3 RNA targets, and Setti *et al.*, in a study of outdoor sampling, reported 20/34 (59%) Particulate Matter (PM) samples were RNA positive for one gene, and 4/34 (11.8%) were positive for two genes (see Table 1). Five studies found no positive samples [Di Carlo P 2020: Dohla M 2020; Kang M 2020, Kwon KS 2020 and Wong JCC 2020].

Three studies reported on two choir practices and potential air transmission. Charlotte N *et al.* followed-up a choir practice in France with 27 participants who attended a choir practice on

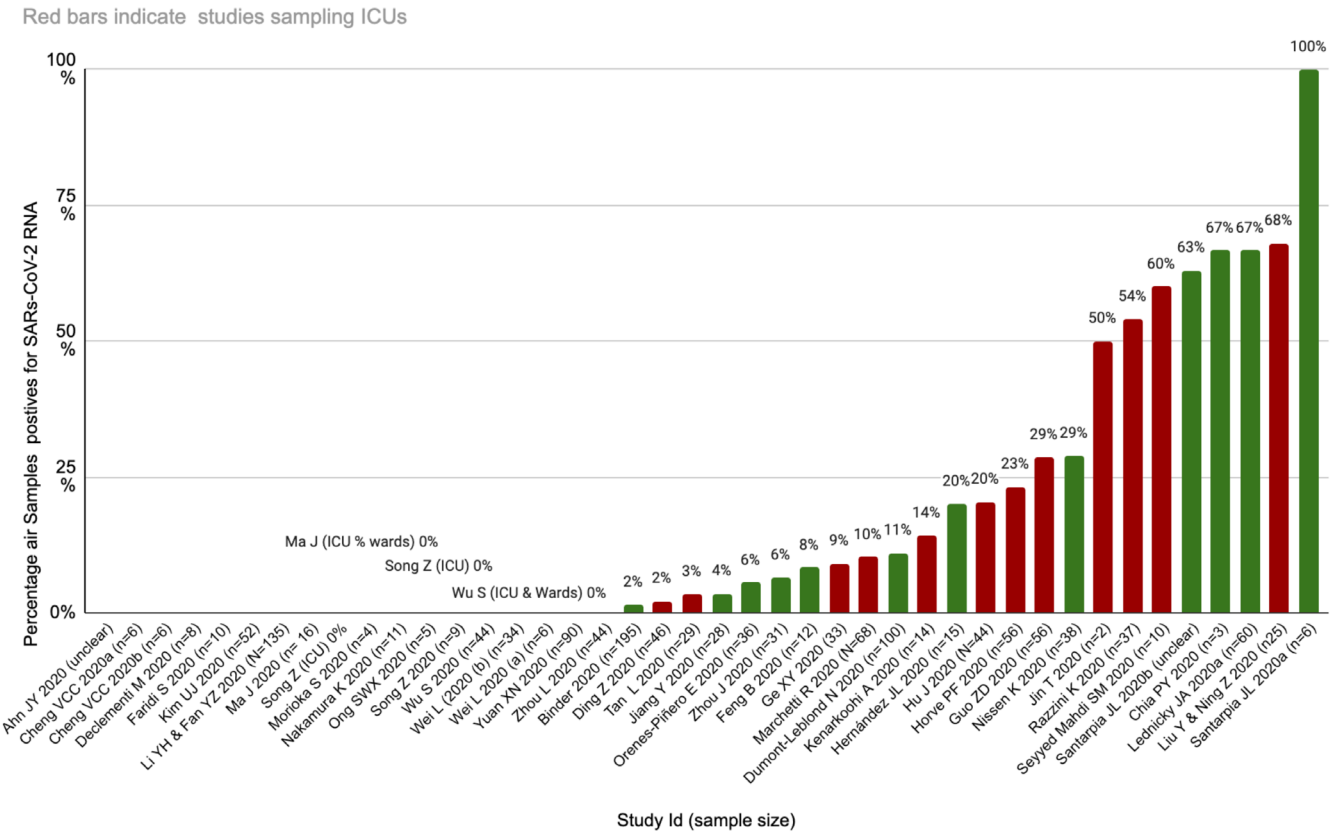

Figure 4. Percentage hospital air samples positive for SARs-CoV-2 RNA (n=42). Red bars indicate studies sampling ICUs.

12 March 2020. Two separate publications [Hamner L 2020 and Miller SL 2020] published on the same Choir Practice Skagit County, Washington, USA. In total, 78 members attended two practices: 87% of choir members subsequently became ill (32 confirmed cases and 20 probable secondary cases).

Viral culture. Ten studies attempted viral culture [Binder 2020, Dohla M 2020, Dumont-Leblond N 2020, Hu J 2020, Lednicky JA 2020a, Lednicky JA 2020b, Nissen K 2020, Santarpia JL 2020a, Santarpia JL 2020b, Zhou J 2020]. In seven of the ten studies, the infectious virus could not be isolated and cytopathic effects could not be observed [Binder 2020, Dohla M 2020, Dumont-Leblond N 2020, Hu J 2020, Nissen K 2020, Santarpia JL 2020b and Zhou J 2020] (see Table 6).

Of the remaining three studies, Lednicky JA 2020b reported that general virus-induced cytopathic effects were observed within two days post-inoculation. The amount of virus present in 390 L of sampled air was low (approximately 340 virus genome equivalents). RT-PCR for SARS-CoV-2 RNA from the cell culture were negative, and three other respiratory viruses were identified: Influenza A H1N1, Influenza A H3N2, and human coronavirus OC43.

Lednicky JA 2020a observed presumed virus-induced CPE for 4/4 RNA-positive hospital air samples. The authors report that plaque assays could not be performed due to a nationwide non-availability of some critical media components in the USA. They also report that it took 6 to 11 days post-inoculation before rounding of the cells was observed with material collected by the air sampler and there is no report of a serial subculture of the positive air samples to demonstrate propagation of a complete replicating virus.

Santarpia JL 2020a reported 3/39 aerosol samples (particle size <1 μm) that cell culture resulted in increased viral RNA at very low levels. An intact virus was observed via transmission electron microscopy in the submicron sample from one room. This study was published as a preprint (checked 5 March 2021) and is subject to methodological criticisms. Serial RT-PCR of cell culture supernatant was unclear and incongruent with the statement that some increase in viral RNA may have occurred.

Table 6. Viral culture methodological issues.

Study	Methodological
Binder 2020	This study separated particles by three sizes: >4 μm, 1–4 μm, and <1 μm and used multiple sampling sites which is a robust sampling methodology. The median day's post symptom was reported as 10 with a range of 1 to 34 days, and only one patient had a cycle threshold for the N gene < 20. This limits the finding of any cultivatable virus and the conclusions.
Hu J	All positive masks were subject to cell culture and inoculated with Vero-E6 cells after blind passage for three generations which is a robust approach. One mask from a critically ill patient was positive for the virus but no details on which passage and at what quantitative burden. The masks could have been contaminated by saliva or nasal secretions and the conclusion stated that masks blocked the release of viable virus in the air exhaled from the patient cannot be confirmed.
Lednicky 2020a	it is not clear why plaque assays could not be performed due to a nationwide non-availability of some critical media components in the US. Three serial 3-hr air samplings were performed. Over the 9 hours, it is likely patients would have moved about and may have been in close proximity to the samplers. The method does not mention particle sizing for the sampler (ie < or > 5 microns) and the sampled particles could be any size and hence it is difficult to state they were true aerosols. No data are provided about health workers who may have been in the room and might have handled the air samplers. Samples were not done at 0.5 m to 1 metre to see if there was a gradient effect. It was noted it took 6 to 11 days post-inoculation before rounding of the cells with material collected by air sampler and there is no report of a serial subculture of the positive air samples to demonstrate propagation of a healthy and propagating virus. Nothings is presented about testing the air sampling isolates in susceptible animal models.
Santarpia JL 2020a and b	For Santapria 2020 (a) we could only find a preprint publication. A large number of samples were collected. Serial PCR of cell culture supernatant was unclear and incongruent with the statement that some increase in viral RNA may have occurred. Increased viral RNA presence is a surrogate and subject to many interpretations and should not be considered equal to the cultivation of replication and infection competent virus on cell culture which was not identified. Western blot assay was not done in cell supernatant samples with non-statistically significant evidence of replication, which would have acted as a control to ensure the findings were not spurious. Western blots are very weak, with no positive control or size markers and the signal doesn't necessarily come from a replicating virus, there's no "before culture" analysis. The presence of virus-like particles on TEM is not proof that these are replicating viruses or necessarily even SAR-CoV-2. No comparisons to control TEM photomicrographs of the live virus from fresh Vero cells are presented to discuss. No size-fractionation techniques were used to determine the size range of SARS-CoV-2 droplets and particles, raising major issues with the statement the data suggests that viral aerosol particles are produced by individuals that have the COVID-19. No information is provided about activity by either patients or the doffing by health workers which may have contributed to hallway air samples being PCR positive. The contamination identified may have accumulated over the extended periods of occupancy and may represent the high frequency of reported PCR positive sites, Floor samples were most heavily reported which supports this finding. The numbers don't match up, Ct values were converted to pseudo TCID50 values based on an equation that obscures what Cts were actually recorded. Reporting 100% or 200% increases in RNA levels is actually only 2–3 fold, and not the way viruses replicate (i.e. exponentially). No plaques were reported to have been detected and no serial passage on subculture was reported. Statistical inferences are very difficult to interpret in Figure 1 based on the error bars. The broad sweeping conclusions that SARS-CoV-2 RNA exists in respired aerosols less than 5 μm in diameter; that aerosols containing SARS-CoV-2 RNA exist in particle modes that are produced during respiration is difficult to justify based on the findings presented. In Santarpia 2020 (b)There are "six patients in five rooms in two wards on three separate days in April of 2020" reported in the text. Table S1 reports are 6 rooms (2 are 7A and 7B and 4 are 5A-D). The abstract reports SARS-CoV-2 RNA was detected in all six rooms – It is therefore not clear whether there are 6 rooms or 5 – One room had 2 patients so the total could be 7 not 6 patients There is no information in the patients and sampling is done 2–24 days post 1st covid test and looks like 4 were sampled less than 3 days post first covid test but there is no information of symptom onset. No ct values were provided on the testing of the pts when first done. A Ct of 45 for E gene is not considered a usual standard and much higher than what most labs use and accept and a lot of background "noise" as a result It is likely an equation as used to calculate the concentration of the virus, however, it is more robust to measure the virus directly than use an equation. EM also does not confirm live virus and does not indicate active viral replication as the authors suggest – where are the comparisons control EM photomicrographs.
Zhou J 2020	No indication any particle size-fractionation techniques were used to determine the size range of droplets and particle differentiation in air sampling. No information on patients is provided and it is possible they were in the later stages of illness when no virus could be reliably cultivated. All surface and air samples from the hospital environment had a Ct value >30, in a range where it is extremely difficult to cultivate the virus. No attempt was made to ensure the sampler was placed at a specific distance from the individuals.
(Wang W, Xu Y 2020 and (Xiao F, Sun J 2020)	Electron microscopy alone does not proof of an infectious virus. Inactivated particles would look the same, and images weren't provided in these studies.

No size-fractionation techniques were used to determine the size range of SARS-CoV-2 droplets and particles. (Table 7 sets out several methodological issues relating to viral culture).

Discussion

We identified 67 primary studies, all were observational and low quality. The results show that RT-PCR RNA can be detected sporadically in airborne samples in a variety of settings. About half the studies did not detect RNA positivity. Some of the reasons for this may be methodological weaknesses in the study design, the lack of validated methods and the location and variable distance of the sampling. There was no clear relationship between the type of setting and positivity of sampling or detectable viral RNA concentrations. The reporting of viral RNA concentrations was heterogeneous as were the sampling methods.

Past attempts to detect infectious particles have proved difficult: aerosols are dilute and culturing fine particles is problematic. In a NEJM editorial, Roy *et al.*, report 'the only clear proof that any communicable disease is transmitted by aerosol came from the famous experiment by Wells, Riley, and Mills in the 1950s, which required years of continual exposure of a large colony of guinea pigs to a clinical ward filled with patients who had active tuberculosis[11].' A 2019 review reported that viral RNA or DNA, depending on the virus, could be found in the air near patients with influenza, respiratory syncytial virus, adenovirus, rhinovirus, and other coronaviruses but rarely reported viable viruses[12]. For coronaviruses, previous evidence supporting the airborne route of transmission is weak[13].

Several studies included in our systematic review and reported in the tables, do not support the airborne transmission hypothesis. An included US study performed active case finding from two index patients and 421 exposed HCWs [Bays D 2020]. Eight secondary infections in HCWs were reported, but despite multiple aerosol-generating procedures, there was no evidence of airborne transmission. No transmission events were found in multiple high-risk exposures from five symptomatic COVID-19 health care workers [Mponponsuo K 2020]; Wong SCY *et al.* reported none of 120 contacts of a patient with initially undetected Covid-19 subsequently became infectious, and Kim UJ *et al.* reported that all 52 air samples were negative for SARS-CoV-2 RNA.

Strengths and limitations

There is a current lack of well-conducted studies addressing airborne transmission: only nine studies identified during the search period reported air sampling outdoors and, in the environment, outside of hospitals. The findings of our review are limited by the low-quality included studies that lack standardised methods, reporting and outcomes. The small sample sizes, the absence of study protocols and the lack of replication further undermine the findings. Sporadic isolation of viral RNA may be due to problems with sampling techniques. Lack of quality is noted across several of the airborne reviews. Furthermore, while our search was comprehensive, it is likely there are studies that we have missed. Our continual updating and scoping of the literature mean we intend to update this review as more studies and evidence become available.

Evidence from the referenced systematic reviews noted the need to improve the quality of evidence. Anderson *et al.* reported the need for further data collection under differing conditions of temperature and humidity[14]. Carducci *et al.* considered no studies had sufficient confirmatory evidence, and only a hypothesis supports airborne transmission[15], Schünemann *et al.* noted direct studies in COVID-19 are limited and poorly reported[16], and Mousavi *et al.* noted the need for rigorous and feasible lines of research in the area of air filtration and recirculation in healthcare facilities[17].

Future studies are warranted to verify findings (particularly including viral culture) before conclusions can be reached about a mode of transmission and important knowledge such as infectious dose. Because of the heterogeneity of the settings, the case-mix limitations, the sampling techniques used clear descriptions and variable study protocols, it is difficult to make meaningful comparisons of air sampling positivity or viral concentrations between settings. Many factors including relative humidity, temperature, aerosolization medium, exposure period, the chemical composition of the air, seasonality, sampling methods, and ultraviolet light exposure can affect the potential infectivity of airborne viruses. While sampling techniques have improved greatly over time, the lack of standardization requires addressing as it limits the development of general recommendations for the sampling of airborne viruses[18].

One essential question is whether observed epidemiologic associations are causal[19,20]. Establishing transmission modes requires integrated epidemiological and mechanistic approaches to narrow uncertainty[21]. Transmission evidence should be context-specific to particular settings (i.e., indoor or outdoor), environment-specific (i.e., the presence of UV light. ventilation etc.) and ensure that exposure an infectious agent has taken place. Identifying those circumstances that promote transmission using all types of relevant evidence that are more likely to promote viral transmission, and therefore, more amenable to intervention.

Methodological issues of the culture methods used, as well as knowledge of the infectiousness of the patient hinder interpretation and suggest that the results should be interpreted with caution. The detection of SARS-CoV-2 RNA in the air cannot presume transmission, since only viable virions can cause disease. No airborne study to date definitively demonstrates SARS-CoV-2 is of an infectious nature, which offers the most robust evidence of transmissibility[22]. CPE alone cannot be relied upon to establish SARS-CoV-2 replication and additional methods are required, including demonstration of viral growth on permissive cell lines, immunofluorescence staining, and confirmed the exclusion of other pathogens or contaminants with sequence confirmation. General virus-induced cytopathic effects were observed in one study, however, RT-PCR tests for SARS-CoV-2 were negative while three other respiratory viruses were identified[23].

Table 7. Live culture results (n=10).

Study (n=64)	Setting	Method	Air Samples positive n/d for SARs-CoV-2 RNA	Live culture	Notes
Binder 2020	Hospital	An observational case series of 20 patients hospitalized with coronavirus disease	3/195 samples from 3 patients	0/3 viable virus	
Dohla M 2020	Quarantined households	An observational study of 43 adults and 15 children living in 21 households; air (also surface and wastewater) samples taken.	0/15	The infectious virus could not be isolated in Vero E6 cells from any environmental sample.	26 of all 43 tested adults were positive by RT-PCR. 10 of 66 wastewater samples and 4/119 surface swab samples were positive for SARS-CoV-2
Dumont-Leblond N 2020	Hospital	An observational study in acute care hospital rooms over the course of nearly two months	11/100 from 6 patient rooms	Viral cultures were negative	
Hu J 2020	Hospital	An observational study: indoor and outdoor air samples in ICUs and CT rooms	aerosol samples 8/38 from ICUs 1/6 from CT rooms samples from medical staff rest areas and corridors were all negative (denominator not clear)	All positive aerosol samples were negative after three passages of Vero-E6 cells inoculated in a blind test.	All positive masks were subjected to cell culture and inoculated with Vero-E6 cells after blind passage for three generations. One mask from a critically ill patient detected positive.
Lednicky JA 2020a	Hospital	Observational: air samples collected, and virus culture attempted	4/4 air samples without a HEPA filter 0/2 samples using a HEPA filter	Virus-induced CPEwas observed for 4/4 RNA-positive air samples.	No other respiratory virus was identified in the samples using a BioFire FilmArray Respiratory 2 Panel. The amount of airborne virus detected per litre of air was small. Plaque assays could not be performed due to a nationwide nonavailability of some critical media components (due to COVID-19 pandemic-related temporary lockdown of production facilities), so TCID50 assays were performed in Vero E6 cells to estimate the percentage of the collected virus particles that were viable. Estimates ranged from 2 to 74 TCID50 units/L of air
Lednicky JA 2020b	Student Healthcare centre	Observational, air samples collected, and virus culture attempted	1/2 air samples	General virus-induced cytopathic effects were observed within two days post-inoculation	Estimated concentration of 0.87 virus genomes L–1 air. The amount of virus present in 390 L of sampled air was low (approximately 340 virus genome equivalents). PCR tests for SARS-CoV-2 vRNA from cell culture were negative. Three respiratory viruses were identified using the Biofire RVP: Influenza A H1N1, Influenza A H3N2, and Human coronavirus OC43

Study (n=64)	Setting	Method	Air Samples positive n/d for SARs-CoV-2 RNA	Live culture	Notes
Nissen K 2020	Hospital	Observational: surface swabs and fluid samples collected, and experimental: virus culture was attempted.	7/19 filter were positive 11 days later, 4/19 were positive for both genes.	No significant CPE was seen after three passages on Vero E6 cells from samples retrieved from ward vent openings or central ventilation ducts or filters	Cycle threshold (Ct) values varied between 35.3 and 39.8 for the N and E gene. Virus culture was attempted: RNA detected in sequential passages but CPE not observed.
Santarpia JL 2020a	Hospital	Observational: size-fractionated aerosol samples collected; experimental: virus culture was attempted.	6/6 patient rooms.	In 3 aerosol samples size <1 μm, cell culture resulted in increased viral RNA. Viral replication of aerosol was observed in the 1 to 4 μm size but did not reach statistical significance.	The presence of SARS-CoV-2 was observed via western blot for all but one of the samples (<1 um, with statistically significant evidence of replication, by rRT-PCR. The intact virus was observed via TEM in the submicron sample from Room.
Santarpia JL 2020b	Healthcare centre	Observational: high-volume (50 Lpm) and low-volume (4 Lpm) personal air samples (& surface samples) collected from 13 Covid-19 patients; experimental: virus culture was attempted.	63% of in-room air samples positive (denominator unclear)	Due to the low concentrations recovered in the samples cultivation of the virus was not confirmed in these experiments. *	. Partial evidence of virus replication from one air sample. In the NBU, for the first two sampling events performed on Day 10, the sampler was placed on the window ledge away from the patients and was positive for RNA (2.42 copies/L of air). On Day 18 in NBU Room B occupied by Patient 3, one sampler was placed near the patient and one was placed near the door greater than 2 meters from the patient's bed while the patient was receiving oxygen (1L) via nasal cannula. Both samples were positive by PCR, with the one closest to the patient indicating a higher airborne concentration of RNA (4.07 as compared to 2.48 copies/L of air).
Zhou J 2020	Hospital	Observational: (air & surface) samples collected from a hospital with a high number of Covid-19 inpatients.	2/31 air samples positive 12/31 suspected	0/14	We defined samples, where both of the PCRs performed from an air or surface sample, detected SARS-CoV-2 RNA as positive, and samples where one of the two PCRs performed from an air or surface sample detected SARS-CoV-2 RNA as suspected

Conclusion

SARS-COV-2 RNA can be detected intermittently by RT-PCR in the air in a variety of settings. A number of studies that looked for viral RNA in air samples found none, even in settings where surfaces were found to be contaminated with SARS-CoV-2 RNA. The lack of recoverable viral culture samples of SARS-CoV-2 prevents firm conclusions to be drawn about airborne transmission. The current evidence is low quality, and there is an urgent need to standardise methods and improve reporting.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

Extended data

Figshare: SARS-CoV-2 and the Role of Airborne Transmission: Systematic review, https://doi.org/10.6084/m9.figshare.14248055.v2[7].

This project contains the following extended data:

- Appendix 1: Updated protocol
- Appendix 2: Search strategy
- Appendix 3: References of included studies
- Appendix 4: Sampling methods

Reporting guidelines

Figshare: PRISMA checklist for 'SARS-CoV-2 and the role of airborne transmission: a systematic review', https://doi.org/10.6084/m9.figshare.14248055.v2[7].

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Open Peer Review

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Reviewer Report 16 April 2021

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✗ **David R. Tomlinson**
University Hospitals Plymouth NHS Trust, Plymouth, UK

Dear Professor Heneghan and team,

I would firstly like to congratulate you for publishing this systematic review on an open access site and for inviting comments. I am grateful for being given the opportunity to respond and to provide peer review. I hope you will consider the points I raise to be in the spirit of the best principles of scientific discourse - i.e., having a focus on methodology and without bias. I also hope that you and your team are open to performing major revisions to your manuscript, after consideration of all comments I provide below, and the other forms of feedback received through open access disclosure of this manuscript. Thank you.

1. Page 4: *'A collection of particles (liquid or solid) ranging in size from 0.001 µm to over 100 mm suspended in a gas defines an aerosol.'*

You have made a typographical error here (easily done!), since aerosols – 'suspensions in air (or a gas) of solid or liquid particles small enough that they will remain airborne for a prolonged period of time because of their low settling velocity' (Tellier R, 2009[1]) – are typically stated as being <100µm diameter, not mm. In addition, the definition of an aerosol typically includes reference to the time over which such particles may remain suspended in the air: would you consider adding this to the definition used in this present manuscript, please? For example, your methods document uses this wording, which is rather more complete in this respect: *'Respiratory droplets <5 µm in diameter are referred to as droplet nuclei or aerosols. Airborne transmission is the spread of an infectious agent caused by the dissemination of aerosols that remain infectious when suspended in air over long distances and time.'* Thank you.

2. Paragraph 2 of your introduction contains two sentences with 55% match to the abstract of Kutter *et al.* (2018) - your reference 4.

This is evidence of presumably accidental plagiarism. The wording should be modified to remedy this please. Thank you.

3. Appendix 7 outlines chosen methodology for excluding studies. Phrases including words such as 'adequately', 'sufficient' and 'clearly defined' are used yet without objective definition provided, introducing the possibility of selection bias.

I would be grateful if you could provide such methodological points in objectively definable terms, please, thereby permitting a more appropriate description as to why each of these studies was ineligible for inclusion. Thank you.

4. Thank you for providing a link to the 'Protocol for a living evidence review (Version 3: 1 December 2020)'. Under *'Study inclusion and exclusion'* is stated: *'Eligible studies should include sampling for the detection of SARs-CoV-2 in the population or the environment on any potential mode of transmission, including droplet, airborne, fomite, orofecal, bloodborne, vertical or other. Studies can be observational including case series, ecological, or prospective; or interventional including randomised trials and clinical reports, outbreak reports, case-control studies, experimental studies, non-predictive modelling. Studies should include sampling for the detection of SARs-CoV-2.'*

Given this description of your intended methods, I am surprised that the methods for the present manuscript state: *'We included field studies that included airborne sampling for SARs-CoV-2 in the population or the environment.'* Ironically, table 3 of Kutter *et al.* (your ref 4) is highly relevant to this important methodological point, since these authors describe the pros and cons of various methods to determine respiratory virus transmission. The cons of air sampling are noted: technical difficulty and possibly only circumstantial level evidence. However, these authors provide a list of further methods usefully employed including virus stability, outbreak (household or hospital) reports, aircraft outbreaks, non-pharmaceutical intervention, experimental infection, air tracer studies and computational modelling / simulation. Each method has its pros and cons, but it is my contention that restricting your present analyses to studies which *'included airborne sampling'* excludes a large body of data which has been the foundation of investigations towards establishing routes of transmission of respiratory viruses amongst humans. Indeed, if your present methods were applied to measles, one would have to conclude that measles is not transmitted via the airborne route since live virus has never been successfully cultured from air samples. Therefore, and in line with this accepted and referenced practice within the field of infectious diseases, it is my contention that your present manuscript should include data from all suitably rigorous* experimental resources and outbreak reports listed here and as described by Kutter *et al*. Thank you.
[*Please forgive my use of a subjective term here: wording would be usefully informed by your response to point (3) I raise, above.]

In case this suggestion seems rather 'obtuse', I would like to draw upon two examples of excluding studies purely on the basis of their laboratory setting and the impact this may have on the validity of any such transmission review.

Firstly, the experiments of van Doremalen *et al.* (2020)[2], in my opinion, represent a particularly valuable contribution towards understanding the possibility of airborne transmission of SARS-CoV-2.

Van Doremalen outline methods:
'Virus stability in aerosols was determined as described previously at 65% relative humidity (RH) and 21-

23°C (Fischer et al., 2016). In short, aerosols (<5 μm) containing HCoV-19 (105.25 TCID50/mL) or SARS-CoV-1 (106.75-7 TCID50/mL) were generated using a 3-jet Collison nebulizer and fed into a Goldberg drum to create an aerosolized environment. Aerosols were maintained in the Goldberg drum and samples were collected at 0, 30, 60, 120 and 180 minutes post-aerosolization on a 47mm gelatin filter (Sartorius). Filters were dissolved in 10 mL of DMEM containing 10% FBS. Three replicate experiments were performed.'

'Viable virus in all surface and aerosol samples was quantified by end-point titration on Vero E6 cells as described previously (van Doremalen et al., 2013).'

Results (extract):
'SARS-CoV-2 remained viable in aerosols throughout the duration of our experiment (3 hours), with a reduction in infectious titer from $10^{3.5}$ to $10^{2.7}$ $TCID_{50}$ per liter of air. This reduction was similar to that observed with SARS-CoV-1, from $10^{4.3}$ to $10^{3.5}$ $TCID_{50}$ per milliliter.'

Conclusions (extract):
'Our results indicate that aerosol and fomite transmission of SARS-CoV-2 is plausible, since the virus can remain viable and infectious in aerosols for hours and on surfaces up to days (depending on the inoculum shed). These findings echo those with SARS-CoV-1, in which these forms of transmission were associated with nosocomial spread and super-spreading events, and they provide information for pandemic mitigation efforts.'

That this study was excluded from your review on the basis of its laboratory setting can only imply that you believe different physical laws might be in operation in a Goldberg drum compared to 'normal air'. However, it is clearly inconceivable that the air within a Goldberg drum using the methods described has unique virus lifespan-enhancing properties. Furthermore, it is biologically implausible that SARS-CoV-2 only ever achieves aerosol **viability** when these same aerosols are created using a Collison nebuliser. Indeed, if the converse was true, you must have reason to believe that physiological aerosol creation during breathing, speech, singing, coughing and/or sneezing uniquely results in immediate (presumably mechanical?) viricidal action. No evidence is presented for this hypothesis, and on the basis of universally applicable physical laws, it is impossible.

Extending this thought process, since WHO IPC Scientific Brief (July 2020) authors (including, I note, co-author TJ on this present manuscript) consider SARS-CoV-2 to be transmitted via close-range *respiratory droplets*, following the logic presented above, for aerosols released from COVID-19 patients to be **non-infectious**, the only mechanism by which SARS-CoV-2 released on respiratory droplets (>5-10μm diameter as per WHO criteria) to be **infectious,** is for SARS-CoV-2 visions to be possessed with the ability to simultaneously measure and move between liberated respiratory particles to ensure that **only those >5-10μm diameter** contain live SARS-CoV-2. Clearly, this is fantasy, since it also [logically] implies that SARS-CoV-2 is sentient and is aware of the current WHO convention for dichotomising respiratory particle size.

Secondly, excluding animal models of transmission not only goes against methods used by Wells and Riley towards the original proof that TB transmission occurs via the airborne route, but suggests that methods employing animal models of infection within strictly controlled environmental conditions are of no use towards understanding human-to-human transmission. It is my contention that – for example – the experiments of Kutter *et al.* (2021) using ferrets

represent a very important contribution to our understanding, providing *'experimental evidence of robust transmission of SARS-CoV-2 via the air'* [3].

I hope you are able to appreciate the important possible harms in excluding such lines of research towards 'understanding the objective nature of reality', and that you are able to provide major revisions to this present manuscript to include all relevant data, as described. Thank you.

5. From this same review article (your ref 4), table 2 states the known transmission routes of SARS-CoV (Coronaviridae) as contact, droplet & aerosol.

As I am sure you are aware, the WHO Ebola 2014 IPC guideline states *'scientists are unaware of any virus that has dramatically changed its mode of transmission'*. So, in light of what is already known about human-to-human Coronaviridae transmission and the potential harms in failing to adequately mitigate every transmission route of SARS-CoV-2, I am curious as to why any infectious disease specialist or team of scientists investigating viral transmission would seek to 'second-guess' the inevitability of its [SARS-CoV-2] airborne transmission? This requires explanation please. Thank you.

6. Following the logic of point (3), your table 3 cannot be interpreted since objectively defined descriptions of 'Quality of included studies' is not provided.

I would be grateful if this analysis of study 'quality' could be updated in line with my suggestion of adopting objective 'quality definitions' above, please. Thank you.

Finally, I do not think it would be appropriate – and I don't want to risk wasting your time in reading yet further comments – for me to undertake any further point-by-point discussion/review of the conclusions which you have drawn from your chosen methods, since it is my contention that your chosen methods are so importantly flawed that the present manuscript should be completely re-written using methods with greater scientific validity, and including the whole range of available data towards SARS-CoV-2 transmission, as described. I hope this seems reasonable.

Many thanks again for providing me with the opportunity to provide peer review. This is a hugely important topic and I sincerely hope you can use comments raised during this process to improve the quality of this manuscript.

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Are the rationale for, and objectives of, the Systematic Review clearly stated?

Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
No

Is the statistical analysis and its interpretation appropriate?
Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: My regular daytime job since 2009 has been as Consultant Cardiologist and Electrophysiologist - perhaps an unlikely job title for anyone reviewing this manuscript. However, as MedRxiv Affiliate since June 2019, I have been exposed to and performing 'release review' of a constant stream of early published works on SARS-CoV-2 - something which has catalysed my interest in this field. I am also experienced in assessing the validity of experimental methods chosen (please see my recent peer reviewed publications and/or preprints) and believe my background allows me to approach this topic without risk of anchoring bias towards one or other mode of respiratory viral transmission. My interest in this area can be further affirmed by evidence of my 'peer review' of the WHO SARS-CoV-2 IPC Scientific Briefing July 2020, assessing the validity of the chosen references *against* airborne transmission of SARS-CoV-2 (my pinned tweet @DRTomlinsonEP). I mention this to illustrate the breadth and depth of my reading and background on this subject, which may otherwise be assumed to be insufficient for someone in my professional role. I hope this is acceptable and that you are able to consider my comments constructively - since this is my intention. Thank you.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.