



SYSTEMATIC REVIEW

REVISED SARS-CoV-2 and the role of airborne transmission: a systematic review [version 3; peer review: 1 approved, 1 approved with reservations, 2 not approved]

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V3 First published: 24 Mar 2021, 10:232
<https://doi.org/10.12688/f1000research.52091.1>
 Second version: 06 Sep 2021, 10:232
<https://doi.org/10.12688/f1000research.52091.2>
 Latest published: 19 Oct 2022, 10:232
<https://doi.org/10.12688/f1000research.52091.3>

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 2020 to 30 May 2022 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 128 primary studies and 29 reviews on airborne SARS-CoV-2. Of the 128 primary studies, 105 (82%) reported data on RT-PCR from air samples, 28 (22%) report cycle threshold values and 36 (28%) copies per sample volume. All primary studies were observational. The research often lacked standard methods, standard sampling sizes and reporting items. We found 69 descriptions of different air samplers deployed. Of the 80 in-hospital studies that reported binary RT-PCR tests, 362/3079 air samples from 75 studies conducted in hospital ward environments were positive (median 8%, IQR=0 to 23%); 23 studies reported 74/703 RT-PCR positive air samples in the ICU setting (median 17%, IQR=0% to 38%) Thirty-eight studies reported potential air transmission in the outdoors or in the community. Twenty-six studies attempted viral culture, none of which definitively demonstrated that replication-competent SARS-CoV-2 could be recovered in the air.

Open Peer Review

Approval Status

	1	2	3	4
version 3 (revision) 19 Oct 2022				 view
version 2 (revision) 06 Sep 2021	 view		 view	
version 1 24 Mar 2021	 view	 view	 view	

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Any reports and responses or comments on the

Conclusion: SARS-CoV-2 RNA is detectable 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 of SARS-CoV-2 from air samples prevents firm conclusions about the definitive role of airborne transmission in SARS-CoV-2.

article can be found at the end of the article.

Keywords

SARs-CoV-2, transmission, COVID, Airborne



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This article is included in the **Pathogens** gateway.



This article is included in the **Coronavirus** collection.

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Competing interests: CH holds grant funding from the NIHR, 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 financial remuneration from an asbestos case and given legal advice on mesh and hormone pregnancy tests cases. He has received expenses and fees for his media work including occasional payments from BBC Radio 4 Inside Health and The Spectator. 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). He has also received income from the publication of a series of toolkit books and for appraising treatment recommendations in non-NHS settings. He is Director of CEBM, an NIHR Senior Investigator and an advisor to Collateral Global. He is also an editor of the Cochrane Acute Respiratory Infections Group 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 favor, he would be entitled to a percentage of the recovery. TJ is coholder of a Laura and John Arnold Foundation grant for 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

Servizi Sanitari Nazionali (AGENAS) of the Italian MoH (2007-2019). TJ is Director Medical Affairs for BC Solutions, a market access company for medical devices in Europe. TJ was 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 for which he receives no funds. He is also an editor of the Cochrane Acute Respiratory Infections Group. TJ is funded to co-author rapid reviews on the impact of Covid restrictions by Collateral Global Organisation. TJ is funded by Oxford University to carry out a living review on the transmission epidemiology of COVID-19 and provide consultancy services to the University. TJ's competing interests are also online <https://restoringtrials.org/competing-interests-tom-jefferson> DHE holds grant funding from the Canadian Institutes for Health Research and Li Ka Shing Institute of Virology relating to the development of Covid-19 vaccines as well as the Canadian Natural Science and Engineering Research Council concerning Covid-19 aerosol transmission. He is a recipient of World Health Organization and Province of Alberta funding which supports the provision of BSL3-based SARS-CoV-2 culture services to regional investigators. He also holds public and private sector contract funding relating to the development of poxvirus-based Covid-19 vaccines, SARS-CoV-2-inactivation technologies, and serum neutralization testing. 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 is a co-investigator on a WHO funded study using integrated human factors and ethnography approaches to identify and scale innovative IPC guidance implementation supports in primary care with a focus on low resource settings and using drone aerial systems to deliver medical supplies and PPE to remote First Nations communities during the COVID-19 pandemic. He also received support from the Centers for Disease Control and Prevention (CDC) to attend an Infection Control Think Tank Meeting. He is a member and Chair of the WHO Infection Prevention and Control Research and Development Expert Group for COVID-19 and a member of the WHO Health Emergencies Programme (WHE) Ad-hoc COVID-19 IPC Guidance Development Group, both of which provide multidisciplinary advice to the WHO, for which no funding is received and from which no funding recommendations are made for any WHO contracts or grants. He is also a member of the Cochrane Acute Respiratory Infections Group. JB is a major shareholder in the Trip Database search engine (www.tripdatabase.com) as well as being an employee. In relation to this work Trip has worked with a large number of organisations over the years, none have any links with this work. The main current projects are with AXA and Collateral Global. He worked on Living rapid literature review on the modes of transmission of SARS-CoV-2 (WHO Registration 2020/1077093-0) and is part of the review group carrying out a scoping review of systematic reviews and meta-analyses of interventions designed to improve vaccination uptake (WHO Registration 2021/1138353-0). AP is Senior Research Fellow at the Centre for Evidence-Based Medicine and reports grant funding from NIHR School of Primary Care Research (NIHR SPCR ESWG project 390 and project 461), during the conduct of the study; and occasionally receives expenses for teaching Evidence-Based Medicine. IJO and EAS have no interests to disclose.

Grant information: The review was funded by the World Health Organization: Living rapid review on the modes of transmission of SARS-CoV-2 reference WHO registration No 2020/1077093. CH, AP and ES also receive funding support from the National Institute of Health Research School of Primary Care Research Evidence Synthesis Working Group project 390 (<https://www.spcr.nihr.ac.uk/eswg>). *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

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How to cite this article: Heneghan CJ, Spencer EA, Brassey J *et al.* **SARS-CoV-2 and the role of airborne transmission: a systematic review [version 3; peer review: 1 approved, 1 approved with reservations, 2 not approved]** F1000Research 2022, 10:232 <https://doi.org/10.12688/f1000research.52091.3>

First published: 24 Mar 2021, 10:232 <https://doi.org/10.12688/f1000research.52091.1>

REVISED Amendments from Version 2

We have updated the review to 30 May 2022. Data were dual extracted, and we assessed quality using a modified QUADAS 2 risk of bias tool. The results now include 128 primary studies and 29 reviews on airborne SARS-CoV-2, and 26 studies attempting viral culture. As a post-hoc analysis, we have also compared the positivity rates of PCR air samples for studies that reported both ICU and non-ICU sample positivity estimates. We have updated the tables and figures with the new studies and added in a meta-analysis of the ICU and non-ICU PCR samples. We have also added further information to the viral culture methodological issues. We have added Jason Oke to the author list for his methodological expertise in this new version.

Any further responses from the reviewers can be found at the end of the article

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 time¹. There are varied definitions of aerosols in the published literature. An aerosol is defined as a collection of particles (liquid or solid) with varying aerodynamic diameters, suspended in the air (gas) for a prolonged time period. The size of the particles and the distance travelled is highly variable and depends on multiple factors including the force generated at the source from which the particles originate, the relative humidity, evaporation level, settling velocity, direction of airflow, the number of air changes per hour, temperature, crowding and other environmental factors²⁻⁵. 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 humidity⁶.

Transmission via droplet nuclei and aerosols in specific settings or situations may potentiate the spread of some viruses in humans, resulting in disease outbreaks that are difficult to manage. The results of several studies investigating airborne human-to-human virus transmission have been largely inconclusive^{7,8}. Among case reports and case clusters for which airborne transmission is hypothesised, published details of the investigations cannot definitively rule out droplet and/or fomite transmission that could also explain human-to-human transmission⁹. Therefore, we aimed to systematically review the airborne transmission evidence for SARS-CoV-2.

Methods

We are undertaking a series of systematic reviews investigating factors and circumstances that impact the transmission of SARS-CoV-2, based on our published protocol last updated Version 4: 1 June 2022) (archived protocol: *Extended data: Appendix 1*¹⁰) Briefly, this review is the third updated version that 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 initially from 1 February 2020 up to 20 December 2020; the searches were updated for version 3 to 30 May 2022 (see *Extended data: Appendix 2* for the search strategies¹⁰). We aimed to include studies that sampled the air for the detection of SARS-CoV-2 in the populations under study or the environment. We primarily included studies that reported sampling for the detection of SARS-CoV-2. However, we also included observational and randomised studies that investigated airborne transmission of SARS-CoV-2. Non-predictive and experimental studies were also considered for inclusion. Studies should include air sampling for the detection of SARS-CoV-2. Studies incorporating models to describe observed data were eligible, but studies reporting solely predictive modelling were excluded. For relevant articles citation tracking was undertaken. We searched the included primary 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 under study or the environment. JB performed the searches, TJ and EAS performed the first screen and CJH checked the initial screening of these studies. Three reviewers (EAS, CJH, TJ) extracted data for each study, and the data was independently checked. 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 m³ of sampled air, 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 sample type. We assessed quality using a modified QUADAS 2 risk of bias tool¹¹. We simplified the tool because the included studies were not primarily reported as diagnostic accuracy studies. Furthermore, there is a lack of high-quality data in published transmission studies¹². We gave particular importance to the description of methods for air sampling and the reporting of sufficient detail to enable replication of the study by other investigators. We examined the following domains: (i) source population – did the study authors adequately describe the source population? e.g., setting, time since symptom onset, presence and degree of symptoms including presence of cough or sneezing, any treatments employed, presence of other mitigating factors, severity of SARS-CoV-2, baseline demographics including concurrent respiratory infections or other comorbidities, distance between study subjects; (ii) methods – did the study authors sufficiently describe the methods used to enable replication of the study? e.g., methods used for diagnosing SARS-CoV-2 in patients, the procedure used for air sampling, time-point for sampling, number of samples per site, cycle threshold determination, culture methods, verification methods to confirm the presence of SARS-CoV-2, airflow/ventilation settings, humidity and any other mitigating

environmental circumstances; (iii) sample sources – did the authors clearly describe the sources for the air samples? What was the volume of air in each sample? Was the period of sampling similar across various sites? (iv) outcome reporting – was the reporting of the results consistent with the study outcomes and was the analysis of the results appropriate – e.g., interval and time-point for testing study participants for potential transmission. The risk of bias for each domain was rated “low”, “moderate” or “high” depending on the adequacy of reporting. One reviewer (EAS) assessed the risk of bias while a second author (CJH) independently verified the risk of bias. Any disagreements were resolved through discussion. Where a consensus could not be reached, a third reviewer (IJO) arbitrated. We summarise data narratively and report the outcomes as stated in the paper, including quantitative estimates when reported and the detection of the culture of SARS-CoV-2, including quantitation, whenever available.

As a post-hoc analysis, we compared the positivity rates of PCR air samples for studies that reported both ICU and non-ICU sample positivity estimates. Using a random-effects model with inverse variance weighted meta-analysis, the difference in positivity rates was computed as odds ratios (OR) with 95% confidence intervals (CI). A statistician (JO) performed the analysis independently before seeing the study data. In a sensitivity analysis, a continuity correction was applied to studies (n=4) where neither arm reported a positive sample.

Results

From 1,001 records screened, we identified 240 eligible studies (see Figure 1; 83 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 studies¹⁰). We included 128 primary studies and 29 reviews (see *Extended data*: Appendix 3 for references to included studies and Table 1 and Table 2 for the characteristics of the included studies¹⁰).

Reviews

We found 29 reviews on SARS-CoV-2: 22 reviews [Anderson EL 2020, Agarwal 2020, Aghalari 2021, Bahl P 2020, Birgand G 2020, Carducci A 2020, Chen PZ 2020, Cherrie JW 2021, Comber L 2020, Dinoi A 2021, Ekram W 2020, Ji B 2020, Mehraeen E 2020, Niazi S 2020, Noorimotlagh Z 2020, Palmer JC 2021, Rahmani 2020, Ribaric NL 2021, Ren Y 2020, Singhal S 2020, and Wilson NM 2020, Vardoulakis S 2021] were about airborne transmission and prevention; four reviews were about airborne transmission and procedures [Goldstein KM 2021, Hussain A 2020, Kay JK 2020, and Schünemann HJ] and three were about 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 ranged from April 2020 up to January 2022. Only nine 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 form¹³.

Quality of included primary studies (n=128)

All included primary studies were observational (some with experimental components) and of low quality (see Table 3).

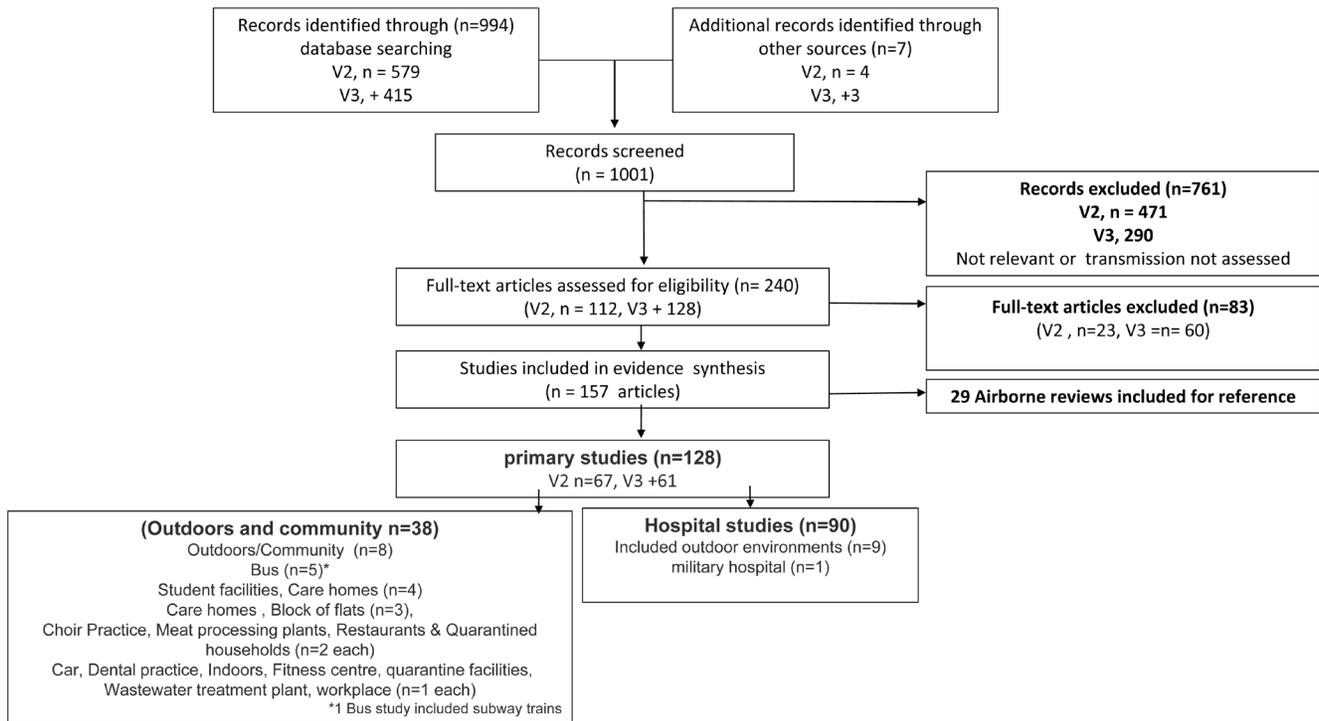


Figure 1. Flow Chart for Airborne Transmission.

Table 1. Study characteristics: primary studies.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Adenaiye OO 2021	University campus and community	USA	COVID-19 cases series. Fomite (phone) swabs, and 30-minute exhaled breath samples	30-minute breath samples while vocalizing into a Gesundheit-II; 2 paired breath samples 1 with and 1 without a mask; 1 or 2 visits 2 days apart.	No mask coarse = 15/78 fine = 22/78 With mask coarse = 10/71 fine = 14/71	All positive aerosol samples were negative after three passages of Vero-E6 cells inoculated in a blind test.
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.
Alkalamoumi H 2021	Hospital	Lebanon	Air samples over 2 consecutive days in the COVID-19 unit hallway, near the staff station, and in patient rooms.	Air samples were collected inside the ED COVID-19 unit using the Coriolis µ microbial air sampler (Bertin Technologies) at a flow rate of 200 L/min for 20 min over two consecutive days.	0/13	Not attempted.
Ang AX 2021	Hospital	Singapore	Air and surface samples were collected from one isolation ward and two open-cohort wards housing laboratory-confirmed COVID-19 patients	Air sampling was conducted with filter-based SASS 3100 air samplers (Research International). The sampler collects total suspended particle (TSP) with no particle size cutoff. The filter media were the default 44 mm diameter SASS bioaerosol filter (polyester material, no electrostatic charge, Research International) with two different pore-sizes.	13/27	0/27
Baboli 2021	Hospital	Iran	Passive and active sampling methods were employed and compared with regard to their efficiency for collection of airborne SARS-CoV-2 virus particles.	Fifty one indoor air samples were collected in two areas, with distances of less than or equal to 1 m (patient room) and more than 3 m away (hallway and nurse station) from patient beds.	6/51	Not attempted.
Baribieri P 2021	Hospital	Italy	Five 24-h PM10 samples in a COVID-19 geriatric ward in late June 2020,	PM10 collected by a low noise (<35 dB) air sampler (SILENT Air Sampler—FAI Instruments S.r.l., Roma, Italy) for 24 h on quartz fiber filters (prefired 47 mm diameter Pallflex, Pall Corporation, Port Washington, New York) with single sampling head operating at a flow rate of 10 L/min with a relative uncertainty of 5% of the measured value. One PM sample (24 h for a total of 14.4 m3 of air) was collected every day.	10/20	patient swabs cultured*
Barksdale AN 2020	Hospital	USA	four air samples were taken in the ED to evaluate SARS-CoV-2 contamination levels	Stationary air samples were collected using a Sartorius Airport MD8 air sampler operating at 30 liters per minute for 30 minutes onto an 80mm gelatin filter.	1–9	Not attempted.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Bays D 2020	Healthcare setting	USA	Two detailed case studies	No sampling performed	Not attempted.	N/A
Bazzazpour S 2021	Dental clinics	Iran	36 air samples at dental clinics	Air sampling was done (n = 36) collecting particulate samples on PTFE filters at flow rates of 30 to 58 L/min.	13/36	Not attempted.
Ben-Shmuel 2020	hospital & quarantine hotel.	Israel	Surface and air sampling were conducted at two COVID-19 isolation units and in a quarantine hotel.	Air sampling was performed using an MD8 air sampler (Sartorius, Göttingen, Germany) equipped with gelatine membranes (3.0 µm filtration cut-off) at 50 L/min sampling rate for 20 min.	2/6 quarantine hotel 1/1	0/3
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
Bokharaei-Sallim F 2021.	hospital	Iran	two air sampling strategies. used simultaneously in three hospital wards	Liquid impaction, an impinger with a standard nozzle was employed to capture virus aerosols in a collecting liquid. Sampling was performed on the 5 mL of DMEM media Air samples were prepared by the flow rate of 1.5 L/min for 180 min. In the filtration view, polytetrafluoroethylene filters by diameter of 25 mm and 0.4 µm porosity (SKC Inc) were used in the 25 mm 2-piece cassettes of clear styrene (SKC Inc)	Liquid impaction 0/7 Filtration 0/7	Not attempted.
Cai Y 2020	Hospital	China	Air samples and 128 environmental surface swabs were collected from 14 patients in 4 departments with temporary COVID-19 ICU wards.	Sample collected using a dry-filter air sampler (52-mm electret filters; InovaPrep ACD-200 Bobcat, America) operating at a speed of 20048L/min for 60 minutes in the 14 temporary ICU wards. The filters were eluted in 7-mL elution fluid (comprising water, a low-concentration surfactant [0.075%49 Tween 20], and a pH buffer [20mM Tris (hydroxymethyl) aminomethane or phosphate-buffered saline]; InovaPrep, America), which was mixed with viral50transport medium (sterile Hank's fluid.	0/15	N/A
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.
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.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
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.
Cheng VCC 2021	Hospital	China	environmental samplings, and whole-genome sequencing (WGS) were performed for a hospital outbreak.	Swab samples from the patients' bedside environments and air grilles (10 cm x 120 cm in size at the ceiling height of 2.35 m in the corridor and 2.6 m in the cubicle) of the air ventilation system in ward 2D were taken for SARS-CoV-2 using RT-PCR testing before and after terminal disinfection	8/22 air grilles	Not attempted.
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 air samples	Not attempted.
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 $D < 0.056 \mu\text{m}$) up to coarse particles ($D > 18 \mu\text{m}$).	Outdoor atmospheric concentrations of SARS-CoV-2 were very small (< 0.8 copies m^{-3})	Not attempted.
Coleman KK 2021	Hospital	Singapore	Exhaled breath emitted by COVID-19 patients	Used a G-II exhaled breath collector, to measure viral RNA in coarse and fine respiratory aerosols emitted by COVID-19 patients during 30 minutes of breathing, 15 minutes of talking, and 15 minutes of singing. Participants were seated facing the truncated cone-shaped inlet, with air drawn continuously (130 L/minute) around the subject's head and into the sampler. Aerosols were collected in 2 size fractions, namely coarse ($> 5 \mu\text{m}$) and fine ($\leq 5 \mu\text{m}$).	25/66 samples	0/25 samples
Conte M 2021	Indoor Community	Italy	air samples collected in different community indoors	(one train station, two food markets, one canteen, one shopping centre, one hair salon, and one pharmacy) in three Italian cities: metropolitan city of Venice (NE of Italy), Bologna (central Italy), and Lecce (SE of Italy). Air samples were collected using quartz fibre filters with low-volume samplers	0/7	N/A

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
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 1.5 l/min.	0/8	Not attempted.
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
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.
de Rooij MMT 2021	Meat processing plant	Holland	SARS-CoV-2 screening of workers operating in cooled production rooms and intensive environmental sampling	Stationary air sampling was performed at potential hotspots based on workers' density and ventilation characteristics in both production rooms. a filter-based technique was used to sample inhalable dust—airborne particles small enough to enter the respiratory tract.	1–12	Not attempted.
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.
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.

Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Dubey A 2021	India	portable air sampling from the medicine ward, intensive care unit, and emergency ward admitting COVID-19 patients.	Total suspended particulate (TSP) air sampler, (M/s. Yayuvodhan, Okhla Industrial Area, New Delhi) which was calibrated as per national standards by CSIR-NPL, India was used for collecting suspended particulate matter from the air.	medicine ward 1m. 6/6; 3m 2/6 ICU 1m. 6/6; 3m 3/6 EmWard 5/6 Nursing station (glass wall) 0/6 Nursing station area ICU (glass wall) 0/6	Not attempted.
Dumont-Leblond N 2020	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
Dumont-Leblond N 2021	Canada	Air and no-touch surfaces of 31 rooms from 7 LTCFs were sampled	Air sampling was performed using an IOM Multi dust sampler (SKC, Eighty Four, PA, USA) loaded with a 3 µm gelatin filter (Sartorius Stedim Biotech, Göttingen, Germany).	0/7	Not attempted.
Dziedzinska R 2021	Czech Republic	Air and surface samples in a Post Office and Shopping Centre	The air was sampled by the commercially available air washer LW220 (Beurer, Ulm, Germany).	0/2	Not attempted.
Escudero D 2021	Spain	presence of SARS-CoV-2 in the air of two ICUs and in the pneumology ward dedicated to the treatment of patients with COVID-19.	The air samples were obtained using two different methods: (1) SAS Bioser Mod. Microbio 0111302 sampling equipment with an air flow of 500 l/300 s and a Rodac plate measuring 55 mm in diameter from which samples were subsequently obtained with pre-humidified swabs. With this system the estimated volume of air passing through the plate in one hour is 5,967 l; and (2) A filtration ramp with a polyethersulfone membrane filter (FLITER-LAB®) of pore size 0.1 µm and measuring 47 mm in diameter, connected to the hospital vacuum system by means of a 60 kPa vacuumeter.	ICU 0/6 Ward 0/1	N/A
Faridi S 2020	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% antifungal 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.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARs-CoV-2 RNA (unless otherwise stated)	Viral culture
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.
Ge XY 2020	Hospital	China	Environmental: 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 Haemodialysis clinic 0/12 fever clinic 0/12 respiratory ward 0/6	Not attempted.
Ghaffari HR 2021	Hospital	Iran	indoor air samples of intensive care unit (ICU) with confirmed COVID- 19 patients and its surroundings.	Detection of SARS-CoV-2 was conducted in the four sections of ICU including the patient section, nurse station, rest room, and doorway of ICU. The low volume sampler (LVS) (ESPS Model, Fanpaya) was applied to collect SARS-COV-2 virus bound to PM2.5 and PM10	2/16 ICU 2/8 Ward 0/8	Not attempted.
Gharehchahi E 2021	Hospital	Iran	Sampling of indoor air, on the surfaces, and the fomites of a COVID-19 referral hospital	Indoor air sampling was conducted utilizing a standard midjet impinger containing 15 ml of viral transfer medium (VTM) equipped with a sampling pump with a flow rate of 10 L min ⁻¹ for 60 minutes.	Total 7/17 ICU 2/3 -ve pressure room 1/1 A&E 1/4 Ward 0/4 CT scan 0/2 Offices 2/2 Laundry 0/1 Temp Waste Storage 1/1	Not attempted.
Gholipour S 2021	Wastewater treatment plant	Iran	analyzed the presence of viral RNA of SARS-CoV-2 in raw wastewater and air samples of WWTPs	A total of 15 air samples were collected using all-glass impingers, containing phosphate buffer solution. Air sampling was performed at three sites in WWTP A, including pumping station and activated sludge plants at a height of 1.5 m above the ground level.	6/15	Not attempted.

Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Gomes da Silva P 2022	Portugal	Air samples from eleven different areas of the Hospital (4 COVID-19 areas)	Two cyclonic microbial air samplers, a Coriolis® µ and a Coriolis® Compact (Bertin Instruments, Montigny-le-Bretonneux, France). Using the Coriolis® µ, three consecutive air samplings were collected from each of the eleven areas of the Hospital for 10 min each with an airflow rate of 100 L/min (total of 1 m3), 200 L/min (total of 2 m3) and 300 L/min (total of 3 m3), respectively. Air samples with the Coriolis® µ were collected on wet medium, with 4 mL of sterile phosphate buffered saline (PBS) added to the collection cones before sampling.	total 2/44 ICU 2/8 COVID-19 ward 0/17 areas non covid 0/19	Not attempted.
Günther T 2020	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.
Guo ZD 2020	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.
Hamner L 2020 and Miller SL 2020	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.
Hamza H 2021	USA	Air samples (< 6ft) and far-field (>6ft) of each patient for 3.5 hours were collected.	Air samples on filter media	17/104	Not attempted.
Hemati et al., 2021	Iran	Air samples (45 SARS-CoV-2, 62 bacteria, and fungi) were collected from different wards	The air samples for virus detection in each ward were collected using the standard midget impinger (SKC, Inc., England) containing 20-mL viral transport medium (VTM) at flow rate of 2 L min ⁻¹ for 4 h (480 L) (Faridi et al., 2020).	6/45 ICU 1/6 patient rooms 2/14 CT scan 1/2 PPE rooms 1/4	Not attempted.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARs-CoV-2 RNA (unless otherwise stated)	Viral culture
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, SWINN) 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.	3/9 in clinic area A and B	Not attempted.
Hoffman JS 2022	public buses	USA	Surveillance sampling in public buses by installing fabric sensors in vehicle air filtration systems.	15 actively deployed buses in the Seattle King County Metro fleet. Collected supplementary pre-filters after more than 7 days of being installed inside the HVAC systems of actively-used metro buses (blue). Also swabbed commonly-touched surfaces on the bus (red).	filters 5/37	Not attempted.
Horve PF 2020 published as Horve PF 2021	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	Not attempted.
Horve PF 2021	Isolation dormitory	USA	Cohort of subjects occupying COVID-19 isolation dormitory and environmental viral load over time, symptoms, and room ventilation	Active air samples were collected using the AerosolSense 2900 sampler (Thermo Scientific, Catalog #121561-00). The AerosolSense sampler works by drawing air into an accelerating impactor at a rate of 200 L/min, causing particles to impact onto a collection substrate.	Unclear	Not attempted.
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-hydrocol 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 analysed. 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.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
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 litres/minute (Merck, Germany).	1/28 air samples	Not attempted.
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.
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.
Kayalar O 2021	Urban	Turkey	Ambient particulate matter (PM) samples in various size ranges were collected from 13 sites including urban and urban background locations and hospital gardens in 10 cities	A total of 155 samples (TSP, n=80; PM2.5, n=33; PM2.5-10, n=23; PM10, n=19) were collected daily using various PM samplers in each city. Samples were collected on glass fibre filters (GF) and Teflon filters (TF) with different sampling equipment Samplers: SKC filter sampler; dichotomous PM sampler; high volume air sampler; low volume stack filter; Zambelli PM sampler; High volume cascade sampler	20/203 positive	Not attempted.
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.		Not attempted.
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.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Kotwa et al., 2021	Hospital	Canada	Air and surfaces samples in rooms of COVID-19 patients	4 bioaerosol samplers were used for sampling the first 45 patients enrolled that were not intubated. For each patient, 1 to 2 different bioaerosol samplers were used in each run. Using an air sampling pump (GilAir Plus Personal Air Sampling Pump, Sensidyne, St. Petersburg, FA), air samples were obtained using the 1-µm pore size, 37-mm polytetrafluoroethylene (PTFE) membrane filters (SKC Inc., Eighty Four, PA), the 37-mm 3-piece cassette with 0.8-µm polycarbonate (PC) filter (Zefon International, Ocala, FL), and 25-mm gelatin membrane filters (SKC Inc.)	3/146	0/3
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 analysed by rRT-PCR test. Air speed and direction at several specified positions were precisely measured using a portable anemometer	0/39 positive	Not attempted.
Lane MA 2020	Hospital	USA	Air samples in an airborne infection isolation room, bathroom, and anteroom of a ventilated patient with COVID-19	Ten NIOSH BC 251 2-stage cyclone samplers were used.9 The NIOSH samplers separated particles into 3 size fractions, which are collected in a 15 mL centrifuge tube (>4 µm fraction), a 1.5 mL centrifuge tube (1–4 µm fraction) and on a filter cassette containing a 37-mm diameter, polytetrafluoroethylene filter with 2 µm pores (<1 µm fraction).	0/28	N/A
Lane MA 2021	Hospital	USA	Air samples in nursing stations and patient room hallways	Eight National Institute for Occupational Safety and Health BC 251 2-stage cyclone samplers were set up throughout 6 units, including nursing stations and visitor corridors in intensive care units and general medical units, for 6 h each sampling period. The NIOSH samplers separate particles into 3 size fractions, which are collected in a 15 mL centrifuge tube (<4 µm), a 1.5 mL centrifuge tube (1–4 µm), and on a filter cassette containing a 37-mm diameter, polytetrafluoroethylene filter with 2 µm pore size (<1 µm).	total 0/528 ICU 0/384 medical unit 0/144	Not attempted.
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 (HEPA) 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.

Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Lednický JA 2020b	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
Lednický JA 2021	USA	screen for SARS-CoV-2 in a car driven by a COVID-19 patient.	The Sioutas Personal Cascade impactor sampler (PCIS) separates airborne particles in a cascading fashion and simultaneously collects the size-fractionated particles by impaction on polytetrafluoroethylene (PTFE) filters). It has collection filters on four: impactor stages (A-D), and an optional after-filter can be added onto a 5th stage (E). The PCIS separates and collects airborne particulate matter above the cut-point of five size ranges: >2.5 µm (Stage A), 1.0-2.5 µm (Stage B), 0.50-1.0 µm (Stage C), 0.25-0.50 µm (Stage D), and <0.25 µm (collected on an after-filter) (Figure 1).	4/5 filter e - equivalent	1/4 Cq 29.65
Lei H 2020	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, < 1 m from the toilet.	Surface and air: 1/218 ICU samples 2/182 isolation ward samples	Not attempted.
Li H 2021	USA	Air and surface samples collected at a fitness centre	Air was collected by four devices (Fig. S1): Viable Virus Aerosol Sampler (VVAS) and BioSpot-VIVAS (Aerosol Devices Inc., Fort Collins, CO) as stationary samplers; and a 47 mm PTFE filter in an in-line holder (Millipore, Bedford, MA) and a NIOSH two-stage cyclone bioaerosol sampler (BC-251) as personal samplers. A 3-h air sampling at 8 L min ⁻¹ was performed during each visit using either the VIVAS or BioSpot-VIVAS with their air intakes positioned ~1.5 m above ground in the centre of the large fitness space on the first floor.	0/21	Not attempted.
Li X 2022	China	COVID-19 outbreak with two fast food employees infected, using environmental SARS-CoV-2 sampling, epidemiological tracing, viral RNA sequence as well as surveillance method.	at the time of the outbreak there were about 20 people) from four different companies (A-D) (Fig. 1(A)) residing in the same employee residence building share the same public toilet, washroom and bath rooms reserved for female and male, respectively. The air samples were collected into 3 mL virus culture liquid (MT0301) (Yocon Biology Inc., Beijing, China) using one cyclone impinger developed by Peking University and commercialized by a company in Beijing (Fig. S2) as reported Li <i>et al.</i> , 2021	3/20 female washrooms n=2	0/3

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
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-Capture-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.
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
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
Linde KJ 2022	Nursing homes	Holland	Air samples in rooms of infected patients.	In every patient room, 6-hr inhalable dust samples were taken using a filtration-based technique at all three locations (Conical Inhalable dust Sampler (CIS), JS Holdings, UK). In addition, one 6-hr two-stage cyclone-based sample with filter back-up was positioned near the feet of the patient when bedridden or at 1.5 meters from the chair of the patient (NIOSH BC 251), as well as a 1-hr impingement-based sampler positioned in proximity of the head of the patient (5ml BioSampler, SKC, UK) The filtration-based sampler was equipped with a 37mm diameter 2.0µm pore-size Teflon filter. The two-stage cyclone-based sampler allowed size-selective sampling and was equipped with two conical tubes (of 15 ml and 1.5 ml) which sample respectively particulates of 1–4µm and >4µm, and a back-up Teflon filter (37 mm diameter and 2.0 µm pore-size Pall incorporated, Ann Arbor, USA) for particulates of <1µm when operated at a flow of 3.5L/min.	Total: 94/213 Positive Oraphangeal Swab 93/184 Negative OPS 1/29	1/10 impingement-based samples n=4, cyclone based n=6 CDC-NIOSH sampler (>4µm size fraction) had lowest Ct of all environmental samples (29.5) and was from the room of the patient with the lowest OPS Ct-value (19.82).
Linillos-Pradillo 2021	Outdoors	Spain	outdoor air samples (on PM10, PM2.5 and PM1).	Three MCV high volume (30 m3 h-1 flow) samplers were collocated with different inlets (Digital DHA-80) for sampling the PM10, PM2.5 and PM1 specific size fractions. Real time particle monitors TEOM 1405DF (™) tapered Element Oscillating Microbalance) and GRIMM™ 1107, validated against the gravimetric reference method, recorded PM10 and PM2.5 and PM1 mass concentration, respectively.	0/18	Not attempted.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
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 cm ² ; 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.
Liu W 2021	Hospital	China	Surface and air samples in the ICU and general wards of three hospitals	An automatic bioaerosol sampler (WB-15, DINGBLUE TECH, Beijing) based on the combination of cyclone separation and impact was adopted to continuously collect air samples for 40 min at a flow rate of 14L min ⁻¹ . Five air samples were collected at about 30cm from the mouth of one corresponding patient who did not wear a surgical mask in the ICU	1/40 ICU 1/9 General Ward 0/5 other 0/16	Not attempted.
López (a) 2021	Hospital	Mexico	Air sampling in patient rooms	A vacuum pump was used to sample the air in three areas of Clinic A and the COVID-19 patients care room of 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, SWINIX) coupled to a vacuum system through a previously disinfected plastic hose (Figure 1), filtering the air with a flow of 9.6 L/min in each filter holder.	3-10	Not attempted.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Lotta-Maria AH 2021	Hospital & Home	Finland	Air and surface samples from the surroundings of 23 hospitalized and eight home-treated COVID-19 patients	<p>Seven different air collection methods were used. A Dekati PM10 cascade impactor (20 l/min air flow) with three stages (> 10, >2.5, and >1 µm), The impactation stages of PM10, PM2.5, and PM1 were fitted with 25-mm-diameter cellulose acetate membrane filters (CA filter, GE Healthcare Life Sciences) and the backup plate with a 40-mm C</p> <p>The BioSpot 300p bioaerosol sampler prototype (Aerosol Devices Inc.)</p> <p>To increase the sample collection rate, the biosampler is equipped with eight wicking tubes fitted with three nozzle jets to secure gentle transfer of the sample.</p> <p>As a more portable solution for personal area air sampling, a standard 25-mm gelatin (Sartorius Stedim Biotech) or mixed cellulose ester (MCE) filter equipped in the Button sampler with a Gillian 5000 air sampling pump, 4 l/min air flow, and a porous curved surface inlet was used</p> <p>Three Andersen cascade impactors (400 W pump and 28.3 l/min flow rate) were used simultaneously</p> <p>a Dekati eFilter was used in two collections. The eFilter monitors changes in real-time particle concentration by utilizing a small diffusion charger powered by an inner chargeable battery.</p>	33/259 samples (12/29 air collections)	0/33
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
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
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.
Mahdi SMS 2021	Hospital	Iran	Air and surfaces of ICU ward in one of the designated hospitals in Tehran	The air sampling was done at a distance of 1.5 to 2 meters from the patient's bed.	44840	Not attempted.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Mallach G 2021	Hospital & Long term care home	Canada	Particulate air sampling in rooms with COVID-19 positive patients in hospital ward and ICU rooms, rooms in long-term care homes experiencing outbreaks, and a correctional facility experiencing an outbreak.	Aerosol (small liquid particles suspended in air) samples were collected onto gelatin filters by Ultrasonic Personal Air Samplers (UPAS) fitted with <2.5µm (micrometer) and <10 µm size-selective inlets operated for 16 hours (total 1.92m ³), and with a Coriolis Biosampler over 10 minutes (total 1.5m ³).	ICU 4/23 Ward 7/92 LTC 3/15 Correctional facility 1/8	0/15
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.
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.
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.
Moharir SC 2022	Hospital & homes	India	Air, samples from different locations occupied by coronavirus disease (COVID-19) patients	Air samples were collected on disposable gelatin filters (Sartorius, Cat. No. 17528-80-ACD) using AirPort MD8 air sampler (Sartorius, Cat. No. 16757). 1000 L of air was collected at a flow rate of 50 L per minute and a sampling time of 20 min.	hospital 40/80 ICU 10/22 non ICU 20/58 pts home 10/18	1/3 in the home setting

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
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.
Morioka S 2020	Hospital	Japan	2 case reports	Air was sampled using an MD8 airscon 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.
Mouchtouri 2020	Hospital, nursing home, LTCF & a ferry	Greece	Air and Surface samples from a ferryboat during a COVID-19 ongoing outbreak investigation and a nursing home and from three COVID-19 isolation hospital wards and a long-term care facility	portable air sampler (Sartorius Airport MD8) with air flow set to 50 L per minute and 10 min sampling time. Gelatin membrane filters of 80 mm diameter (Sartorius 17528-80-ACD) were used.	1/12 air samples	Not attempted.
Mponponsoo 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.
Nagle S 2022	Hospital	France	air and surface contamination in the rooms of patients with COVID-19 in the acute phase of the disease.	Air sampling of 600 litres in 6 minutes at 1 and 3 meters,	7/59	Not attempted.
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 airscon 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.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Nannu Shankar S 2021	Apartments	USA	Air and surfaces in bedrooms of two 20-year-old persons with symptomatic COVID-19 were sampled self-isolating persons.	Using polytetrafluoroethylene (PTFE) filters and a Viable Virus Aerosol Sampler (VVAS), (2) size-fractionated particles in aerosols according to aerodynamic size using a 2-stage cyclone aerosol sampler (NIOSH bioaerosol sampler, BC-251) and a Sioutas personal cascade impactor sampler (PCIS), The PCIS (catalog no. 225-370, SKC Inc., US) was used with a Leland Legacy pump (catalog no. 100-3002, SKC Inc., US) and operated at a flow rate of 9 L/min for 90 min. PTFE filters (25 mm, 0.5 µm pore, catalog no. 225-2708, SKC Inc., US) were used to collect particles of size >2.5 µm, 1-2.5 µm, 0.5-1 µm and 0.25-0.5 µm in the 4 collection stages.	Volunteer A NIOSH 1/3 PTFE 0/3 Volunteer B NIOSH 4/6 PCIS 4/10	volunteer B Oct 2 4/8* Oct 6 0/8
Nor 2021	Hospitals	Malaysia	Fine indoor air particulates with a diameter of ≤ 2.5 µm (PM2.5) was collected over four weeks during 48-h measurement intervals in four separate hospital wards	Air purifier (FANFIL AP510M, Aire-plus Technology, Singapore) was deployed at ~ 1 m distance in wards C and D, ~ 8 m in ward B, and no air purifier in single occupant room.	2-4	Not attempted.
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
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
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.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Ong SWX 2021	Hospital & Community		Air samples from airborne-infection isolation rooms and a community isolation facility housing COVID-19 patients	Air samples were collected using a BioSpot-VIVAS BSS300-P bioaerosol sampler (Aerosol Devices, Fort Collins, CO), which collects airborne particles using a water-vapor condensation method into a liquid collection medium at a flow rate of 8 L per minute.	6-12	0/6
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 m ³ /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.
Pan J 2022	Student rooms	USA	collected surface swab samples and heating, ventilation, and air conditioning (HVAC) filters from 24 rooms that had been occupied by students who tested positive for COVID-19,	collected HVAC filters from each room, if available, cut them into ~3 cm x 8 cm pieces, and stored them at -80 °C. swabbed the air exhaust grilles in the public bathrooms in the quarantine dormitory.	15/21 HVAC 4/6 bathroom exhaust grilles	Culture samples with a Ct value < 33, and none contained culturable virus.
Passos RG 2021	Hospital and community	Belo Horizonte BRAZIL	Environmental and hospital air sampling from May to August 2020	62 samples from two hospitals with different occupancy and public plazas, bus stations/terminals, and hospital areas, with a large circulation and concentration of people. “The epidemiological situation during this monitoring period suggested an accelerated spread of the virus in the city”	5/62 (ICU 3/22) ward areas 2/20	Not attempted.
Pivato A 2021	Environmental	Padua, Veneto, Italy	Remote sampling of PM from outdoor environmental stations	10 outdoor sites were sampled from 23 Feb to 8 March 2020 before national lockdown. A total of 44 PM 2.5 and 5 samples were taken	0/44	Not attempted.
Pochtowyi AA 2021	Hospital	Russia	Pilot study of the presence of SARS-CoV-2 in aerosol samples and surface swabs from different locations in the respiratory infection department and ICUs of the First Infectious Diseases Hospital in Moscow	Air and surface samples collected from rooms of PCR and clinically diagnosed C19 patients in the two departments. Graphics in the paper show sampling sites and results. Samples taken from floors, corridors; handles, beds, nurses stations, cafeteria etc of patients	5/15 (5/6 ICU samples, 0/9 other areas)	Not attempted.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
<p>Ramuta MD 2022</p>	<p>Community setting in Wisconsin and Minnesota</p>	<p>USA</p>	<p>Observational study assessing whether active air samplers can be used for prospective air surveillance of SARS-CoV-2 in real-world congregate settings between July 19, 2021, to February 9, 2022</p>	<p>527 air samples from 15 different locations such as coffee shops and sports facilities. In total, nine samples with RdRp Ct-values ranging from 19.8 to 30.2 were selected for SARS-CoV-2 whole-genome sequencing, of which six OPS, one cyclone-based sample, one filtration-based sample and one surface swab.</p>	<p>106/527 52 inconclusive.</p>	<p>Not attempted.</p>
<p>Razzini K 2020</p>	<p>Hospital</p>	<p>Italy</p>	<p>Observational; 5 air (& 37 surface) samples collected in the ICU for Covid-19 patients.</p>	<p>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).</p>	<p>20/20 from the contaminated area 0/8 semi-contaminated 0/9 clean areas.</p>	<p>Not attempted.</p>
<p>Ruffina de Sousa 2022</p>	<p>Hospital</p>	<p>Sweden</p>	<p>sample air from rooms occupied by COVID-19 patients in a major hospital.</p>	<p>Room air was collected using the Tuberculosis Hotspot detector (THOR) electrostatic air sampler. Ten different patient rooms with adjoining anterooms were sampled in the above way.</p>	<p>patient rooms 9/22; adjoining anterooms 10/22</p>	<p>PFU recovery patient room 3/9; anteroom 8/10</p>
<p>Santarpia JL 2020a</p>	<p>Hospital</p>	<p>USA</p>	<p>Size-fractionated aerosol samples collected; virus culture was attempted.</p>	<p>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.</p>	<p>6/6 patient rooms.</p>	<p>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.</p>
<p>Santarpia JL 2020b</p>	<p>Healthcare centre</p>	<p>USA</p>	<p>High-volume (50 Lpm) and low-volume (4 Lpm) personal air samples (& surface samples) collected from 13 Covid-19 patients; virus culture was attempted.</p>	<p>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. Samples collected using a Sartorius Airport MD8 air sampler operating at 50 Lpm for 15 min.</p>	<p>63% of in-room air samples positive (denominator unclear)</p>	<p>Cultivation of virus was not confirmed in these experiments.</p>

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Schoen CN 2022	Medical centre/ maternity wing	USA	Case series of 6 term mothers who tested positive up to 7 days before SVD. 5/6 wore masks throughout labour and delivery. Study took place between May 2020 and January 2021.	Two samplers were used: 1 at the bedside, midway between the subject's head and hips at about 4 feet high and 2 was located 6–10 feet from the subject's head, ~5 feet high.	0/12	Not attempted.
Semelka CT 2021	Academic hospital	North Carolina US	To assess effect of mask on viral spread two 30 minute sampling runs were undertaken. One with COVID patients without a mask followed by a run with the patient wearing a mask.	59 adults with Covid 19 and comorbidities aged around 58 yrs. provided 20 samples each: 9 samples from both environmental sampling runs (3 stations with 1 surface sample and 2 pooled samples from air sampling devices), the patient mask, and the initial NP swab.	2/52.	Not attempted.
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 fibre 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 m ³ 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.
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.
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.
Stern RA 2021 (a)	Mid sized hospital in Boston	USA	Simultaneous air sampling in five sites six times in the period 29 April to 22 May 2020. N gene PCR probe	Cascade samplers were located at floor height: (1) outside the entrance to a COVID-19 ward (CW1); (2) in a personal protective equipment (PPE) donning room outside the entrance to another COVID-19 ward (CW2); (3) outside the entrance to the medical intensive care unit (ICU); (4) at a staff workstation in the emergency department (ED); and (5) at a nursing staff workstation of a ward not designated for care of COVID-19 patients (NCW)	8/90 6 difference time points; 5 different sampling areas ICU: 2/18 ED: 2/18 Covid Ward: 1/36 Non CW: 3/18	Not attempted.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Stern RA 2021 (b)	30 locations in a hospital and a COVID-19 quarantine facility.	Kuwait	210 air samples collected simultaneously over two periods: April 30 - May 20, 2020 and June 24 - July 10, 2020	Samples from ICUs, nurses' workstations, the rooms of inpatients with and without symptoms, observation rooms for the ED, locker rooms, bathrooms, a lobby, waiting areas, patient hallways, swab testing areas, and outside hospital entrances.	13/210	Not attempted.
Song Z 2020	Public Health Clinical Centre	China	Observational surveillance to evaluate the risk of viral transmission in AIIRs with 115 rooms in three buildings at the Shanghai Public Health Clinical Centre, 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.
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. Samples were collected with a cascade sampler running continuously for 48 hours collecting fine ($\leq 2.5 \mu\text{m}$ aerodynamic diameter), coarse ($2.5\text{--}10 \mu\text{m}$) and large ($\geq 10 \mu\text{m}$) particles	1/29 0/17 clean areas 1/12 patient rooms*	Not attempted.
Thuresson S 2022	Hospital	Sweden	Observational study carried out Skåne, southern Sweden from March 20 to April 21 to assess variables associated with SARS-CoV-2 in the air: patient characteristics, distance from patient, room ventilation, and supportive treatment with a focus on potential AGPs.	Air samples were taken for 10 minutes, several times a week, in 3 infectious disease wards, 4 ICUs, 3 medical wards modified into COVID-19 units, and 1 ED. Patient records were examined: PCR and Ct were recorded.	26/310; 22/231 within patient rooms	Not attempted.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Vosoughi M 2021	Hospital	Iran	Samples of air were taken from respiratory section-1 (COVID-19), laboratory section, CT section, respiratory section-2 (COVID-19), respiratory section-1 (COVID-19) check-up room, respiratory section-2 (COVID-19) station section, emergency section, and ICU. Samples were taken 2 to 5 m from beds and at different heights (1 to 2 mt). Map provided in paper.	32 samples taken from areas with 55 SARS-CoV-2 positive patients and 35 HCWs	0/32	Not attempted.
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 13:00 pm 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 litres/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.
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.
Winslow R 2021	Hospital	UK	Prospective observational study of 30 low SATS Covid-19 cases who received either supplemental oxygen, CPAP or HFNO (10 in each arm). The study took place between 11/12/2020 and 19/02/2021	NP swab, plus 3 air and 3 surface samples taken from each ppt and the clinical environment. Air samples were taken with a Coriolis micro air sampler. Recruitment was opportunistic. PCR was carried out with ORF1a and N genes probes.	4/90	1/51 nasopharyngeal sample

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
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.
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.
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
Yarahmadi R 2021	ICU	Iran	Sampling stations were located around various parts of ICU as described in Figure 1	20 air samples taken around ICU from 3 zones: patient breathing zone, general area, breathing zone of health care personnel.	4/20 2/4 patient breathing zone 1/8 general area; 1/8 HCW breathing zone	Not attempted.
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.
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.
Zhang X 2022	Non clinical areas of University buildings	University of Michigan, US	Observational study to assess air and surface contamination, relating it to the epidemiological situation and estimating the risk of infection with SARS-CoV-2	Between August 2020 and April 2021, areas in classrooms, rehearsal rooms, office areas, cafeterias, buses, gyms, student activity buildings and heating, ventilation and air-conditioning (HVAC) system tunnels were wet swabbed (surfaces) or air sampled. Results were linked to University dashboard for linkage with case incidence	4/256 (1.6%) air samples and 4/517 (1.5%) surface samples	Not attempted.

	Setting	Country	Method	Samples Source	Air Samples PCR positive for SARS-CoV-2 RNA (unless otherwise stated)	Viral culture
Zhou J 2020	Hospital	UK	Observational: (air & surface) samples collected from a hospital with a high number of Covid-19 inpatients.	<p>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).</p>	<p>2/31 air samples positive 12/31 suspected</p>	0/14
Zhou L 2020	Hospital	China	Study of collected samples of exhaled breath of patients ready for discharge and air samples.	<p>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.</p> <p>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.</p>	0/44	Not attempted.

Table 2. Study characteristics: reviews.

Study Id (n=29)	Fulfils systematic review methods	Research question (search date up to)	No. included studies	Main results	Key conclusions
Airborne transmission (n=22)					
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.
Aghalari Z 2021	yes	To evaluate the SARS-COV-2 transmission through indoor air in hospitals and its prevention practices (search December 2019 to October 1, 2020).	11 studies included in qualitative synthesis	Analysis of the articles showed that Asian countries (Iran, China, Singapore) were more concerned with the SARS-COV-2 transmission through hospital air. Four articles did not confirm SARS-COV-2 in the air, but seven articles reported the SARS-COV-2 from air samples.	Several factors can affect the positive or negative SARS-COV-2 detection in air samples, such as environmental conditions in hospitals, sampling methods, sampling height and distance from patients, flow rate and sampling time, efficiency and functionality of ventilation systems, use of disinfectants.
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 2020)	4 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)	10 papers	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.

Study Id (n=29)	Fulfills systematic review methods	Research question (search date up to)	No. included studies	Main results	Key conclusions
<p>Birgand G 2020 and Birgand G 2020/JAMA</p>	<p>no</p>	<p>Evidence for airborne contamination of SARS-CoV-2 in hospitals (search conducted to 21 Jul y repeated on October 27, 2020 for JAMA publication)</p>	<p>17 articles</p>	<p>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.</p>	<p>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.</p>
<p>Carducci A 2020</p>	<p>no</p>	<p>To describe the state of the art of coronavirus airborne transmission (search conducted 5 June)</p>	<p>64 papers classified into three groups: laboratory experiments (12 papers), air monitoring (22) and epidemiological and airflow model studies (30)</p>	<p>Airborne transmission of SARS-CoV-2 was suggested by studies across the three groups, but methods were not standardised.</p>	<p>No studies had sufficient confirmatory evidence, and there is only a hypothesis to support airborne transmission</p>
<p>Chen PZ 2020</p>	<p>yes</p>	<p>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)</p>	<p>64 studies (n = 9,631 total specimens)</p>	<p>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,</p>	<p>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</p>

Study Id (n=29)	Fulfils systematic review methods	Research question (search date up to)	No. included studies	Main results	Key conclusions
Cherrie JW 2021	No	To summarise the reported SARS-CoV-2 RNA air and surface contamination concentrations in workplace settings where the virus is present, particularly considering the quality of the methods used, to draw lessons for future methodological developments (up to the 24th December 2020)	35 papers were reviewed: three were available as preprints and the remainder as peer-reviewed publications	Typically, around 6% of air and surface samples in hospitals were positive for SARS-CoV-2 RNA, although there is very limited data for non-healthcare settings. <ul style="list-style-type: none"> The quality of the available measurement studies is generally poor, with little consistency in the sampling and analytical methods used. Few studies report the concentration of SARS-CoV-2 in air or as surface loading of virus RNA, and very few studies have reported culture of the virus. The best estimate of typical air concentrations in health care settings is around 0.01 SARS-CoV-2 virus RNA copies/m³ 	The reliability of the reported data is uncertain. The methods used for measuring SARS-CoV-2 and other respiratory viruses in work environments should be standardised to facilitate more consistent interpretation of contamination and to help reliably estimate worker exposure.
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.
Dinol A 2021		Identification/quantification of SARS-CoV-2 RNA in airborne samples comparing different sites: outdoor sites, indoors in hospitals and healthcare settings, and community indoor locations. (Start of COVID-19 pandemic until 31/08/2021)	73 published papers on experimental determination of SARS-CoV-2 RNA in air	11.7% of studies are in outdoor, 75.3% in hospitals, and 13% in community public indoors. <ul style="list-style-type: none"> Average positivity rate was larger in hospital compared to outdoors and public indoor sites. Contamination of surfaces was more frequent than air but with a lower positivity rate. SARS-CoV-2 RNA concentrations in air follows outdoors < public indoors < hospitals. 	Concentrations of SARS-CoV-2 RNA in air were highly variables and, on average, lower in outdoors compared to indoors. Among indoors, concentrations in community indoors appear to be lower than those in hospitals and healthcare settings.
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.

Study Id (n=29)	Fulfills systematic review methods	Research question (search date up to)	No. included studies	Main results	Key conclusions
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 <i>et al.</i> , 2020; Kim <i>et al.</i> , 2020; Masoumbeigi <i>et al.</i> , 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.

Study Id (n=29)	Fulfills systematic review methods	Research question (search date up to)	No. included studies	Main results	Key conclusions
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.
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.
Palmer JC 2021 & Duval D 2022	yes	To evaluate the potential for long distance airborne transmission of SARS-CoV-2 in indoor community settings and to investigate factors that might influence transmission. (search 1 Jan 2020 to 19 Jan 2022)	22 reports relating to 18 studies	Long distance airborne transmission was likely to have occurred for some or all transmission events in 16 studies and was unclear in two studies (GRADE: very low certainty). In the 16 studies, one or more factors plausibly increased the likelihood of long distance airborne transmission, particularly insufficient air replacement (very low certainty), directional air flow (very low certainty), and activities associated with increased emission of aerosols, such as singing or speaking loudly (very low certainty). In 13 studies, the primary cases were reported as being asymptomatic, presymptomatic, or around symptom onset at the time of transmission.	Authors suggest long distance airborne transmission of SARS-CoV-2 might occur in indoor settings such as restaurants, workplaces, and venues for choirs, and identified factors such as insufficient air replacement that probably contributed to transmission
Ribaric NL 2021	Yes	Assessed the nature and extent of air- and surface-borne SARS-CoV-2 contamination in hospitals to identify hazards of viral dispersal and enable more precise targeting of infection prevention and control. (Until June 2021)	51 observational cross-sectional studies comprising 6258 samples were included.	SARS-CoV-2 RNA was detected in one in six air and surface samples throughout the hospital and up to 7.62 m away from the nearest patients. The highest detection rates and viral concentrations were reported from patient areas. The most frequently and heavily contaminated types of surfaces comprised air outlets and hospital floors. Viable virus was recovered from the air and fomites.	The nature and extent of hospital contamination indicate that SARS-CoV-2 is likely dispersed conjointly through several transmission routes, including short- and long-range aerosol, droplet, and fomite transmission.

Study Id (n=29)	Fulfills systematic review methods	Research question (search date up to)	No. included studies	Main results	Key conclusions
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	Analysis of different papers on mode of transmission it was found that this virus is highly contagious and spreads through air droplet, close contact, through fomites and different metallic surfaces and through aerosol in surroundings with high aerosol generating procedures only.	Results demonstrate the fact that early screening, social distancing, isolation of symptomatic patients, respiratory etiquette are the main armaments presently to deal with this virus till effective treatment or vaccine becomes available in the near future.
Vardoulakis S 2021	No	Review of the environmental sampling, laboratory, and epidemiological studies on viral and bacterial infection transmission in washrooms (Search dates 2000-2020)	38 studies from 13 countries	A wide range of enteric, skin and soil bacteria and enteric and respiratory viruses were identified in public washrooms, potentially posing a risk of infection transmission.	Although there is a risk of microbial aerosolisation from toilet flushing and the use of hand drying systems, we found no evidence of airborne transmission of enteric or respiratory pathogens, including COVID-19, in public washrooms.
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=4)					
Goldstein KM 2021	Yes	Risk of viral transmission during nebulizer treatment of patients with coronavirus disease 2019 (search updated to Sep 1 2020)	22 articles: 1 systematic review, 7 cohort/case-control studies, 7 case series, and 7 simulation-based studies. Eight individual studies involved patients with SARS, five involved MERS, and one involved SARS-CoV-2.	one study found with COVID19 patients - Heinzerling <i>et al.</i>	Specific evidence that exposure to nebulizer treatment increases transmission of coronaviruses similar to COVID-19 is inconclusive.
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.

Study Id (n=29)	Fulfills systematic review methods	Research question (search date up to)	No. included studies	Main results	Key conclusions
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).	45 studies 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)	6 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
Adenaiye OO 2021	Yes	Yes	Yes	No	Not Applicable
Alkalamouni H 2021	Unclear	Unclear	Yes	Yes	Not Applicable
Ahn JY 2020	Yes	Yes	No	Unclear	Not Applicable
Ang AX 2021	Unclear	Yes	Yes	No	Not Applicable
Baboli 2021	Unclear	Yes	Yes	No	Not Applicable
Baribieri P 2021	Unclear	Yes	Yes	Yes	Not Applicable
Barksdale AN 2020	Unclear	Unclear	Yes	No	Not Applicable
Bays D 2020	Yes	Yes	Not Applicable	Yes	Yes
Bazzazpour S 2021	Unclear	Yes	Yes	Yes	Not Applicable
Ben-Shmuel 2020	Unclear	Yes	Yes	Yes	Not Applicable
Binder 2020	Yes	Yes	Yes	Yes	Yes
Bokharaei-Salim F 2021.	Unclear	Yes	Unclear	Yes	Not Applicable
Cai Y 2020	Unclear	Unclear	Yes	Yes	Not Applicable
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
Cheng VCC 2021	Yes	Yes	Yes	No	Not Applicable
Chia PY 2020	Yes	Yes	Yes	Yes	Not Applicable
Chirizzi D 2020	Not Applicable	Yes	Yes	Yes	Not Applicable
Coleman KK 2021	Yes	Yes	Yes	No	Not Applicable
Conte M 2021	Yes	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
de Rooij MMT 2021	Yes	Yes	Yes	Yes	Not Applicable
Ding Z 2020	Yes	Yes	Yes	Unclear	Not Applicable
Döhla M 2020	Unclear	Yes	Yes	Unclear	Not Applicable
Dubey A 2021	Yes	Yes	Yes	Unclear	Not Applicable
Dumont-Leblond 2020	Yes	Yes	Yes	Yes	Not Applicable
Dumont-Leblond N 2021	Unclear	Yes	Yes	Unclear	Not Applicable
Dziedzinska R 2021	Yes	Yes	Yes	Yes	Not Applicable
Escudero D 2021	Yes	Unclear	Yes	Unclear	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
Faridi S 2020	Yes	Yes	Yes	Yes	Not Applicable
Feng B 2021	Yes	Yes	Yes	Yes	Not Applicable
Ge 2020	Unclear	Yes	Yes	Yes	Not Applicable
Ghaffari HR 2021	Unclear	Yes	Yes	No	Not Applicable
Gharehchahi E 2021	Unclear	Yes	Yes	Unclear	Not Applicable
Gholipour S 2021	Unclear	Yes	Yes	No	Not Applicable
Gomes da Silva P 2022	Unclear	Yes	Yes	Yes	Not Applicable
Günther T 2020	Yes	Yes	Yes	Unclear	Yes
Guo ZD 2020	Yes	Yes	Yes	Yes	Not Applicable
Hamner & Miller 2020	Yes	Yes	Not Applicable	Unclear	Yes
Hamza H 2021	Unclear	Unclear	Unclear	Yes	Not Applicable
Hemati <i>et al.</i> , 2021	Yes	Yes	Yes	Unclear	Not Applicable
Hernández JL 2020	Unclear	Yes	Yes	Yes	Not Applicable
Hoffman JS 2022	Unclear	Unclear	Yes	Yes	Not Applicable
Horve PF 2020 & Horve PF 2021	Unclear	Unclear	Yes	Unclear	Not Applicable
Horve PF 2021	Yes	Yes	Yes	Unclear	Unclear
Hu J 2020	Unclear	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
Kayalar O 2021	Unclear	Yes	Yes	Unclear	Not Applicable
Kenarkoochi A 2020	Yes	Yes	Yes	Unclear	Not Applicable
Kim UJ 2020	Yes	Yes	Yes	Yes	Not Applicable
Kotwa <i>et al.</i> , 2021	Yes	Yes	Yes	Yes	Not Applicable
Kwon KS 2020	Yes	Yes	Not Applicable	Yes	Yes
Lane MA 2020	Yes	Yes	Yes	Yes	Not Applicable
Lane MA 2021	Unclear	Yes	Yes	Yes	Not Applicable
Lednický JA 2020a	Yes	Yes	Yes	Unclear	Not Applicable
Lednický JA 2020b	Yes	Yes	Yes	Unclear	Not Applicable
Lednický JA 2021	Yes	Yes	Yes	Yes	Not Applicable
Lei H 2020	Yes	Yes	Yes	Yes	Not Applicable
Li H 2022	Unclear	Yes	Yes	Unclear	Not Applicable
Li X 2022	Unclear	Yes	Yes	Unclear	Unclear
Li YH & Fan YZ 2020	Yes	Yes	Yes	Yes	Not Applicable
Li Y & Qian H 2020	Yes	Yes	Not Applicable	Yes	Yes

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
Lin G 2020	Yes	Yes	Not Applicable	Yes	Not Applicable
Linde KJ 2022	Unclear	Yes	Yes	Yes	Not Applicable
Linillos-Pradillo 2021	Unclear	Yes	Yes	Yes	Not Applicable
Liu Y, Ning Z 2020	Yes	Yes	Yes	Yes	Not Applicable
Liu W 2021	Unclear	Yes	Unclear	Yes	Not Applicable
López 2021	Unclear	Yes	Unclear	Unclear	Not Applicable
Lotta-Maria AH 2021	Yes	Yes	Yes	Unclear	Not Applicable
Lu J 2020	Yes	Unclear	Not Applicable	Unclear	Not Applicable
Luo K 2020	Yes	Yes	Not Applicable	Yes	Yes
Ma J 2020	Unclear	Yes	Yes	Unclear	Not Applicable
Mahdi SMS 2021	Unclear	Unclear	Yes	Unclear	Not Applicable
Mallach G 2021	Unclear	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
Moharir SC 2022	Unclear	Yes	Yes	Unclear	Not Applicable
Moreno 2020	Not Applicable	Yes	Yes	Yes	Not Applicable
Morioka S 2020	Yes	Yes	Yes	Unclear	Not Applicable
Mouchtouri 2020	Unclear	No	Yes	Unclear	Not Applicable
Mponponsuo K 2020	Yes	Yes	Not Applicable	Yes	Yes
Nagle S 2022	Yes	Yes	Yes	Yes	Not Applicable
Nakamura K 2020	Unclear	Yes	Yes	Yes	Not Applicable
Nannu Shankar S 2021	Yes	Yes	Yes	Yes	Not Applicable
Nissen K 2020	Yes	Unclear	Yes	Unclear	Not Applicable
Nor 2021	Unclear	Unclear	Unclear	Unclear	Not Applicable
Ogawa Y 2020	Yes	Yes	Yes	Yes	Yes
Ong SWX 2020	Yes	Yes	Yes	Yes	Not Applicable
Ong SWX 2021	Yes	Yes	Yes	Yes	Not Applicable
Orenes-Piñero E 2020	Yes	Yes	Not Applicable	Yes	Not Applicable
Pan J 2022	Unclear	Yes	Yes	Yes	Not Applicable
Passos RG 2021	Unclear	Yes	Yes	Not Applicable	Not Applicable
Pivato A 2021	Unclear	Yes	Yes	Yes	Not Applicable
Pochtovyi AA 2021	Unclear	Yes	Yes	Yes	Not Applicable
Ramuta MD 2022	Unclear	Yes	Yes	Yes	Not Applicable
Razzini K 2020	Yes	Yes	Yes	Yes	Not Applicable
Ruffina de Sousa 2022	Unclear	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
Santarpia JL 2020a	Yes	Yes	Yes	Unclear	Not Applicable
Santarpia JL 2020b	Yes	Yes	Yes	No	Not Applicable
Schoen CN 2022	Yes	Yes	Yes	Unclear	Not Applicable
Semelka CT 2021	Yes	Yes	Yes	Unclear	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
Stern RA 2021 (a)	Unclear	Yes	Yes	Unclear	Not Applicable
Stern RA 2021 (b)	Yes	Yes	Yes	Unclear	Not Applicable
Song Z 2020	Unclear	Yes	Yes	Yes	Not Applicable
Tan L 2020	Yes	Yes	Yes	Unclear	Not Applicable
Thuresson S 2022	Yes	Yes	Yes	Unclear	Not Applicable
Vosoughi M 2021	Unclear	Yes	Yes	Yes	Not Applicable
Wei L 2020a	Yes	Yes	Yes	Yes	Not Applicable
Wei L 2020b	Yes	Yes	Yes	Yes	Not Applicable
Winslow R 2021	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
Yarrahmadi R 2021	Yes	Yes	Yes	No	Not Applicable
Yuan XN 2020	Unclear	Unclear	Unclear	Unclear	Not Applicable
Zhang D 2020	Yes	Unclear	Yes	Unclear	Not Applicable
Zhang X 2022	Unclear	Yes	Yes	No	Yes
Zhou J 2020	Yes	Yes	Yes	Yes	Not Applicable
Zhou L 2020	Yes	Yes	Yes	Yes	Not Applicable
Total	73	111	101	67	12
	128	128	128	128	128
Percentage	57.0%	86.7%	78.9%	52.3%	9.4%

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 them in 87% of studies (see Figure 2), the research often lacked standard methods, standard sampling sizes and standard reporting. In 57% of the studies, the sample sources were clear, however, outcomes that aimed to demonstrate the

detection of culturable, replicable viruses were lacking. The variation in sample methods coupled with flaws in the reporting made it difficult to distinguish between aerosol and droplet nuclei transmission routes. Interpretation was further limited by the variability in reporting of patient distance from the sampler, use of protective equipment or oxygen masks by patients, time since symptom onset, patient activities

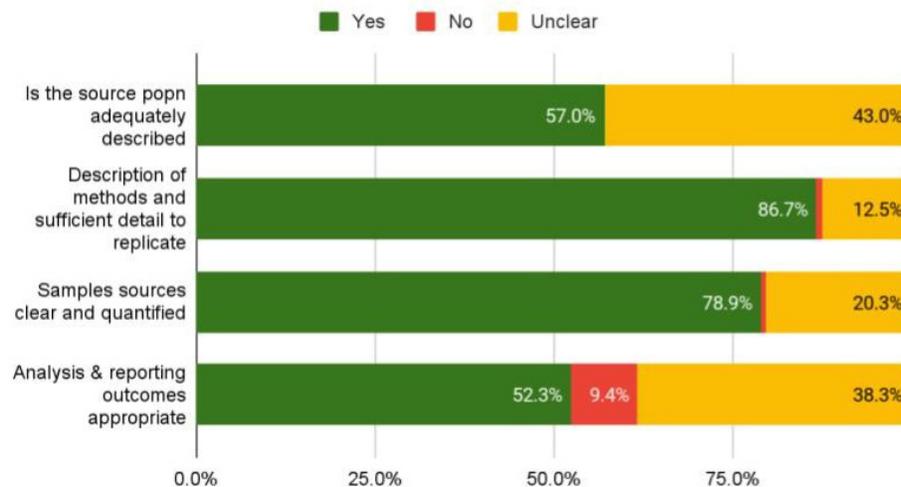


Figure 2. Risk of Bias Airborne Transmission Studies (n=128).

(coughing and sneezing during sampling time), air movement, air conditioning sampler design, method of sampling, storage, and transfer conditions.

Primary studies

We included 128 primary studies, of which 105 (82%) reported binary data on RT-PCR air samples (see Table 1). All the studies were observational. Twenty-eight studies (22%) reported Ct values and 36 studies (28%) reported copies per sample volume (see Table 4).

Of the 128 included studies, 54 (42%) reported viral RNA concentrations (see Table 3). Of these, 31 reported data on Ct and 36 on genome copies. The lack of standardized reporting prevents the pooling of the data. Thirteen studies reported both Ct and genome copies [de Rooij MMT 2021, Dumont-Leblond 2020, Guo ZD 2020, Kayalar O 2021, Lednicky JA 2020a, Lednicky JA 2020b, Lednicky JA 2021, Ma J 2020, Mallach G 2021, Nannu Shankar S 2021, Nor 2021, Passos RG 2021, and Pochtovyi AA 2021]. Only eight studies reported air samples with a RT-PCR Ct below 30: Ang AX 2021, Dubey A 2021, Guo ZD 2020, Linde KJ 2022, Mallach G 2021, Nannu Shankar S 2021, Ramuta MD 2022, Razzini K 2020. We found five studies that reported Cts below this threshold: Dubey A 2021, Guo ZD 2020, Nannu Shankar S 2021, Ramuta MD 2022, and Razzini K 2020. Infectivity (defined by virus growth in Vero cell culture) has been found to be more likely when the RT-PCR Ct value is <25.¹⁴

Table 5 shows 24 studies reporting the size of detectable particles containing RNA from SARS-CoV-2 [Adenaiye OO 2021, Baboli 2021, Baribieri P 2021, Binder 2020, Chia PY 2020, Chirizzi D 2020, Coleman KK 2021, Feng B 2020, Hernández JL 2020, Kayalar O 2021, Lednicky JA 2021, Linde KJ 2022, Liu Y & Ning Z 2020, Lotta-Maria AH 2021, Mallach G 2021, McGain F 2020, Nannu Shankar S 2021, Ong SWX 2021, Passos RG 2021, Semelka CT 2021+, Santarpia 2020a,

Stern RA 2021a, Stern 2021b and Zhang X 2022]. Overall, the methods used for air sampling were heterogeneous across studies. SARS-CoV-2 RNA was detectable in a range of air sample sizes from <1 μm through to >18 μm . Thirteen studies detected particles below <4 μm , and Chirizzi D 2020 *et al.* reported on coarse particles up to a diameter > 18 μm . Different samplers in the same study also detected different size particles. For example, McGain F 2020 *et al.* reported that the APS detected larger aerosols (> 0.37 μm) and MiniWRAS smaller particles (0.01–0.35 μm).

We found 69 different descriptions of air samplers deployed: the two most frequently used samplers were the MD8 sampler, Sartorius, Goettingen, Germany (n=12 studies) and the National Institute for Occupational Safety and Health (NIOSH) Aerosol sampler (n=10 studies). Several studies used different methods, and there were variations in the flow rate used and associated methods that affect sampling techniques (see *Extended data: Appendix 5*¹⁰).

Hospital/Health Center. There were 90 studies conducted in healthcare settings: Of these, 362/3079 air samples in hospital ward environments from 75 studies (median 8%, IQR=0% to 23%) and 74/703 (median 17%, IQR=0% to 38%) air samples in the ICU setting from 23 studies reported RT-PCR positive results. (See Figure 3).

Twenty studies reported sampling results in the hospital environment (non-ICU) and the ICU. Figure 4 shows that ICU environments were approximately twice as likely to detect SARS-COV-2 RNA in air samples, OR 2.07 (95% CI, 1.23 - 3.47, $I^2=0\%$, n = 20 studies, 1300 air samples).

We found eleven studies conducting air sampling both in hospitals and in other environments. Ben-Shmuel *et al.* sampled within the hospital environment and in a quarantine hotel. Lotta-Maria *et al.* sampled the air and surfaces from the

Table 4. Concentrations of PCR samples recovered. Of the 128 included studies, 54 (42%) reported viral concentrations (see Table 3). Of these, 31 reported data on cycle threshold and 36 on genome copies. The lack of standardized reporting prevents the pooling of the data. Thirteen studies reported both cycle threshold and genome copies: de Rooij 2021, Dumont-Leblond 2020, Guo 2020, Kayalar 2021, Lednicky 2020a, Lednicky 2020b, Lednicky 2021, Ma 2020, Mallach 2021, Nannu Shankar 2021, Nor 2021, Passos 2021, and Pochtowl 2021). Eight studies reported air samples with a cycle threshold below 30: Ang 2021, Dubey 2021, Guo 2020, Linde 2022, Mallach 2021, Nannu Shankar 2021, Ramuta 2022, Razzini 2020. Infectivity (defined by virus growth in VERO cell culture) is highly likely when the RT-PCR Ct value is <25. [reference Jefferson *et al.*] We found five studies that reported CTs below this threshold: Dubey 2021, Guo 2020, Nannu Shankar 2021, Ramuta 2022, and Razzini 2020.

Study	Cycle Threshold (Ct)	Copies per m ³ (or L)
Ang AX 2021	E-gene: 29.55–37.22 N-Gene: 34.30–38.95	
Baboli 2021		2.53 - 4.86 copies/m ³
Baribieri P 2021	19th 20th & 21st June range 36.7–38.3 22nd, 23rd June 06/20 negative or > 40	
Ben-Shmuel 2020	Ventilated patient: 34.1 Nurse station: 38.8 Quarantine hotel: 35	
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 – 3.38×10^3 copies per m ³
Chirizzi D 2020		<0.8 copies m ³ for each size range.
Cheng VCC 2021	33.2–38.0	
Coleman KK 2021		Activity N gene copies per expiratory activity Breathing (30 mins): 63.5–550 Talking (15 mins): 79.9–4336 Singing (15 mins): 135–5821
de Rooij MMT 2021	38	5×10^2 copies/m ³
Ding Z 2020		RNA copies for weakly positive sample not calculated.

Study	Cycle Threshold (Ct)	Copies per m ³ (or L)
Dubey A 2021	Ward: <u>1m.</u> <u>3m</u> E gene: 16.1–32.1 21.1–29.7 RdRp-gene: 16.1–29.4. 29.9–34.1 ICU: <u>1m.</u> <u>3m</u> E gene: 19.1–30.2 29.9–32.5 RdRp-gene: 16.8–30.3 30.5–33.7 Emergency Ward In the centre E gene: 26.7–30.2 RdRp-gene: 24.1–34.0 Nursing station separated by glass wall E gene: -ve, RdRp-gene: -ve	
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	Mean 201 genomes /m ³ (range 9.9 to 514)
Feng B 2020	36.5 - 37.8	<1 μm: 1,111 copies/m ³ >4 μm: 744 copies/m ³
Ge XY 2020		
Gomes da Silva P 2022		ICU: 60 min sampling (flow rate 50 L/min) N1 gene 6000 copies/m ³ N2 gene 6575 copies/m ³ First 10 min (flow rate 100 L/min) N1 6362.5 copies/m ³ N2 6662.5 copies/m ³
Guo ZD 2020	Indoor air near air outlet: 35.7 Near patients: 44.4 Near the doctor's office: 12.5	Indoor air near the air outlet: 3.8/L near the patients: 1.4/L near the doctor's office: 0.52/L
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 ×10 ⁴ 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
Kayalar O 2021	RdRp-gene 34.7 to >45 N gene 35.1 to >45	N gene 9917 - 43790 uL ⁻¹ 80 – 504 copy numbers on the filters
Kenarkoohi A 2020	Around 38 for ORF1ab Around 35 for n gene	
Lednicky JA 2020a	36.0, 37.7, 37.4, 38.7 (mean Cq 37.5)	2.82E+03, 9.12E+02, 1.15E+03, 4.68E+02 genome equivalents/25 μL,
Lednicky JA 2020b	39.1	0.87 virus genome equivalents L ⁻¹
Lednicky JA 2021	33.5–40.1	1.24E+03 - 3.14E+04

Study	Cycle Threshold (Ct)	Copies per m ³ (or L)
Lei H 2020	Near the head of the patient Ct 41.25.	
Linde KJ 2022	Range from 29.5 to 37.2	
Lotta-Maria AH 2021		COVID-19 ward Active sampling Range 534–6608 cm ⁻³ (3380 ± 2320 cm ⁻³), Passive sampling 1 sample 3.56 x 10 ³ copies/ml.
Liu Y & Ning Z 2020		ICU: range- 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, 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
Mahdi SMS 2021		Highest RNA concentrations were observed between beds 6 and 7: 3913 copies/ml.
Mallach G 2021	N gene range 30.2– 38.0, mean 35.5 (SD 2.1) E gene range 27.0– 36.9, mean 33.6 (SD 2.3) Ct E gene (range) ICU 33.0 (31.2-34.3) Ward 35.0 (33.3-36.89) Long term Care (LTC) 3968.3 (27.0-35.0) Correctional Facility 32.4	Mean RNA copy numbers E gene 941.6 copy numbers/mL (range 61.3–11,462; SE 752.4) mean RNA concentration in the air 1202.4 copy numbers/m ³ (63.8–11939.9; SE 977.2); Copy numbers mean (range) ICU 224.8 (71.6-529) Ward 134.3 (61.3-276.0) LTC 3968.3 (89.0-11462.3) Correctional Facility 378.9
Moreno T 2020		Genome count range IP2: 14 to 446/m ² for IP2, IP4: 9 to 490/m ² E subway 5 to 378/m ² : 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).
Nagle S 2022	1m: median 38 (range 37–40) 3m: 40 (range 39–42)	

Study	Cycle Threshold (Ct)	Copies per m ³ (or L)
<p>Nannu Shankar S 2021</p>	<p><u>Patient A:</u> <i>NIOSH sampler</i>: 38.2 <u>Patient B: Oct 2</u> <i>PCIS sampling</i> RdRp gene: 16.0–18.0 N gene 14.6–16.8 <i>NIOSH sampler</i> RdRp gene: 16.0–18.0 18.5–32.0 N gene: 17.1–31.1 <u>Patient B: Oct 6</u> <i>PCIS</i>: RdRp gene, N gene -ve <i>NIOSH</i>: RdRp gene: -ve N gene: 37.7</p>	<p>Patient A: GE/cm³ of air <i>NIOSH sampler</i>: 0.06 Patient B: Oct 2 <i>PCIS sampling</i> RdRp gene: 3.01×10^4 - 1.19×10^5 N gene: 6.84×10^4 - 3.04×10^5 <i>NIOSH sampler</i> RdRp gene: 9.89×10^2 - 6.36×10^4 N gene: 2.54×10^3 - 1.68×10^5 Patient B: Oct 6 <i>PCIS</i>: RdRp gene, N gene: -ve <i>NIOSH</i>: RdRp gene: -ve N gene: 0.16</p>
<p>Nissen K 2020</p>	<p>Ct N gene: 35.3 Ct E gene 33.2 Ward 1 specimen Ct 33.0 for E gene only.</p>	
<p>Nor 2021</p>	<p><40</p>	<p>Ward A: 74 ± 117.1 copies μL^{-1} General Ward B: 10 ± 7.44 copies μL^{-1} 179 to 2,738 copies/m³</p>
<p>Ong SWX 2021</p>		
<p>Orenes-Piñero E 2020</p>	<p>Ct from surfaces > 10 cycles of those obtained from the patient, indicating viral load was lower in the room environment.</p>	
<p>Pan J 2022</p>		<p>Quarantine rooms Average 31 copies/m³ (Range 0.3 to 115) Isolation rooms Average 3 copies/m³ (0.2 to 24)</p>
<p>Passos RG 2021</p>	<p>32–34</p>	<p>genomic units m³ 0.19 -66.4</p>
<p>Pochtovyj AA 2021</p>	<p>Close to detection limit: 38–40</p>	<p>28.1 to 140.6 copies per/m³</p>
<p>Ramuta MD 2022</p>	<p>Emergency housing facility: 25.9–31.8 Brewery Taproom: 30.0–42.9</p>	
<p>Razzini K 2020</p>	<p>ICU: Mean Ct 22.6 Corridor: Mean Ct 31.1</p>	
<p>Ruffina de Sousa 2022</p>	<p>Average Ct Patient rooms: 38.3 Anterooms 38.3 Air exhaust vent in the patient room: 33.5 Air exhaust vent in the anteroom: 33.0</p>	

Study	Cycle Threshold (Ct)	Copies per m ³ (or L)
Santarpia JL 2020a		Concentrations up to around 7.5 TCID 50 /m ³ of air.
Santarpia JL 2020b		In-room air samples mean 2.42 copies/L of air NBU Room A (Patient 1) 2.42 copies/L NBU Room B (Patient 3), Near the patient: 4.07 copies/L >2 m from the patient's bed: 2.48 copies/L Outside rooms in hallways: 2.51 copies/L. Highest concentrations in NBU while a patient was receiving oxygen through a nasal cannula (19.17 and 48.22 copies/L).
Seyyed Mahdi SM 2020		Highest RNA concentrations observed between beds 6 and 7 (3,913 copies per ml)
Stern RA 2021 (a)		Range 7–51 Highest concentrations in ED, May 13–15: 51 copies/m ³ 2nd highest at Non-Covid Ward, May 11–13: 47 copies/m ³
Stern RA 2021 (b)		Outside hospital gates: 3–17 copies/m ³ Symptomatic patient rooms: 8–25 copies/m ³ ICUs: 18–21 copies/m ³ Outdoors, Gate 7: 17 copies/m ³
Thuresson S 2022		In patient rooms median concentration: 115 copies/m ³ (IQR 31 to 232)
Winslow R 2021	Ct values for positive and suspected-positive air samples were substantially higher than paired samples in the nasopharynx, indicating minimal viral RNA in the air.	
Zhang D 2020		Range 285 to 1,130 copies/m ³ . Inside adjusting tank 285 copies/m ³ and 603 copies/m ³ . 5 m from Hospital outpatient building 1,130 copies/m ³ , 5 m from the inpatient building undetected
Zhang X 2022		Gym: weight room: 15/10/20 (sample time 257 mins) 6.00 × 10 ⁻² gc/L, Gym: weight room: 30/10/20 (253 mins): 2.80 × 10 ⁻² gc/L Gym: weight room 2/8/21 (242 mins): 7.60 × 10 ⁻² gc/L Bus: passenger area 18/11/20 (72 mins): 2.30 × 10 ⁻² gc/L Gym: weight room in Fall: 2.80 × 10 ⁻² gc/L Gym: weight room Fall & Winter: 6.00 × 10 ⁻² gc/L
Zhou J 2020		101 to 103 copies of SARS-CoV-2 RNA in all air samples; no significant difference between sample areas.

Table 5. The size of air particles in the sample. Twenty-four studies reported detecting RT-PCR SARS-CoV-2 test positive RNA in a wide range of sizes (see Table 4).

Study	Samples Source	Size of air particles
Adenaiye OO 2021	30-minute breath samples while vocalizing into a Gesundheit-II, 2 paired breath samples 1 with and 1 without a mask; 1 or 2 visits 2 days apart.	Coarse (> 5 µm) 25/149 Fine (≤ 5 µm) 24/149
Baboli 2021	Fifty-one indoor air samples were collected in two areas, with distances of less than or equal to 1 m (patient room) and more than 3 m away (hallway and nurse station) from patient beds.	PM1, PM2.5, and PM10 detected
Baribieri P 2021	PM10 was collected by a low noise (<35 dB) air sampler (SILENT Air Sampler—FAI Instruments S.r.l., Roma, Italy) for 24 h on quartz fibre filters.	PM ₁₀
Binder 2020	Eight National Institute for Occupational Safety and Health (NIOSH) BC 251 Aerosol Samplers 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	Aerosol particle size <4 µm
Chia PY 2020	Air sampling was performed in three of the 27 airborne infection isolation rooms (AIIRs). Bioaerosol samplers were 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)
Coleman KK 2021	Used a G-II exhaled breath collector to measure viral RNA in coarse and fine respiratory aerosols emitted by COVID-19 patients during 30 minutes of breathing, 15 minutes of talking, and 15 minutes of singing. participants were seated facing the truncated cone-shaped inlet, with air drawn continuously (130 L/minute) around the subject's head and into the sampler. Aerosols were collected in 2 size fractions, namely coarse (>5 µm) and fine (≤ 5µm).	All three activities Coarse fraction: 14.6% Fine fraction: 85.4%
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, and 12 bedside air samples)	RNA was detected in the air sample in <1 µm and >4 µm fractions,
Hernández JL 2020	Air was 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.
Kayalar O 2021	A total of 155 samples were collected daily using various PM samplers in each city. Samples were collected on glass fiber filters (GF) and Teflon filters (TF) with different sampling equipment Samplers: SKC filter sampler; dichotomous PM sampler; high volume air sampler; low volume stack filter; Zambelli PM sampler; High volume cascade sampler	The PM sizes of positive samples were PM _{<0.49} (n = 1), PM _{0.49-0.95} (n = 1), PM _{0.95-1.5} (n = 1), and PM _{>1.5} (n = 2).
Lednický JA 2021	The Sioutas Personal Cascade impactor sampler (PCIS) separates airborne particles in a cascading fashion and simultaneously collects the size-fractionated particles by impaction on polytetrafluoroethylene (PTFE) filters). It has collection filters on four impaction stages (A–D), and an optional after-filter can be added to a 5th stage (E). The PCIS separates and collects airborne particulate matter above the cut-point of five size ranges: >2.5 µm (Stage A), 1.0–2.5 µm (Stage B), 0.50–1.0 µm (Stage C), 0.25–0.50 µm (Stage D), and (Stage E) <0.25 µm (collected on an after-filter).	PCIS filter A Cq value: 36.66 PCIS filter B: 35.23 PCIS filter C: 34.37 PCIS filter D: 33.50 PCIS filter E <0.25: 40.1

Study	Samples Source	Size of air particles
Linde KJ 2022	In every patient room, 6-hr inhalable dust samples were taken using a filtration-based technique at all three locations (Conical Inhalable dust Sampler (CIS), JS Holdings, UK). In addition, one 6-hr two-stage cyclone-based sample with filter back-up was positioned near the feet of the patient when bedridden or at 1.5 meters from the chair of the patient (NIOSH BC 251), as well as a 1-hr impingement-based sampler positioned in proximity of the head of the patient (5ml BioSampler, SKC, UK) The filtration-based sampler was equipped with a 37mm diameter 2.0µm pore-size Teflon filter. The two-stage cyclone-based sampler allowed size-selective sampling and was equipped with two conical tubes (of 15 ml and 1.5 ml), which sample respectively particulates of 1–4µm and >4µm, and a backup Teflon filter (37 mm diameter 2.0 µm pore-size Pall incorporated, Ann Arbor, USA) for particulates of <1µm when operated at a flow of 3.5L/min.	>4 µm: 60% 1–4 µm 50% <1 µm 20% Inconclusive and positive results were more frequently present in the largest particle size fraction,
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 were 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 were collected using 80-mm-diameter filters packed into a holder with an effective deposition area of 43.0 cm ² ; 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 m ⁻³ in the 0.25–0.5 µm and 0.5–1.0 µm range, respectively. By contrast, aerosols in the supermicrometre region were mainly observed in the PPAR of zone C of Fangcang Hospital with concentrations of 7 copies/m ³
Lotta-Maria AH 2021	"Seven different air collection methods were used. A Dekati PM10 cascade impactor (20 l/min air flow) with three stages (>10, >2.5, and >1 µm), The impaction stages of PM10, PM2.5, and PM1 were fitted with 25-mm-diameter cellulose acetate membrane filters (CA filter, GE Healthcare Life Sciences) and the backup plate with a 40-mm C The BioSpot 300p bioaerosol sampler prototype (Aerosol Devices Inc.) As a more portable solution for personal area air sampling, a standard 25-mm gelatin (Sartorius Stedim Biotech) or mixed cellulose ester (MCE) filter equipped in the Button sampler with a Gilian 5000 air sampling pump, 4 l/min airflow, and a porous curved surface inlet was used Three Andersen cascade impactors (400 W pump and 28.3 l/min flow rate) were used simultaneously a Dekati eFilter was used in two collections.	SARS-CoV-2 RNA was detected in the following particle sizes: 0.65–4.7 µm, >7 µm, >10 µm, and <100 µm.
Mallach G 2021	Aerosol (small liquid particles suspended in air) samples were collected onto gelatin filters by Ultrasonic Personal Air Samplers (UPAS) fitted with <2.5µm (micrometer) and <10 µm size-selective inlets operated for 16 hours (total 1.92m3), and with a Coriolis Biosampler over 10 minutes (total 1.5m3).	RNA samples were positive in 9.1% (6/66) of samples obtained with the UPAS 2.5µm samplers, 13.5% (7/52) with the UPAS 10µm samplers, and 10.0% (2/20) samples obtained with the Coriolis samplers.
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).
Nannu Shankar S 2021	Using polytetrafluoroethylene (PTFE) filters and a Viable Virus Aerosol Sampler (VIVAS), (2) size-fractionated particles in aerosols according to aerodynamic size using a 2-stage cyclone aerosol sampler (NIOSH bioaerosol sampler, BC-251) and a Sioutas personal cascade impactor sampler (PCIS). The PCIS was used with a Leland Legacy pump and operated at a flow rate of 9 L/min for 90 min. PTFE filters (25 mm, 0.5 µm pore) were used to collect particles of size >2.5 µm, 1–2.5 µm, 0.5–1 µm and 0.25–0.5 µm in the 4 collection stages.	virus-associated particles were >0.25 µm and >0.1 µm respectively
Ong SWX 2021	Air samples were collected using a BioSpot-VIVAS BSS300-P bioaerosol sampler (Aerosol Devices, Fort Collins, CO), which collects airborne particles using a water-vapor condensation method into a liquid collection medium at a flow rate of 8 L per minute.	SARS-CoV-2 nucleic acid was detected in aerosols <1 µm, 1–4 µm, and >4 µm in diameter.

Study	Samples Source	Size of air particles
Passos RG 2021	Two types of aerosol samples in indoor environments were collected: (1) aerosol samples of suspended particles using air samplers with filters, in order to quantify the concentrations of SARS-CoV-2 in aerosols and to estimate the size of airborne particulates. In this case, the lower limit was estimated by the filter porosity and the upper limit defined by a cyclone separator (<4 µm at a flow rate of 2.5 L min ⁻¹ ; or with no cyclone, no upper size limit), and/or by approximate comparison between results of sampling with different filters (pore sizes), at the same location; and (2) aerosol deposition samples, in order to determine the deposition rate of airborne SARS-CoV-2.	Air samples tested positive for SARS-CoV-2, in particle sizes >4 µm and 1–4 µm in diameter. Samples from the fractionated size <1 µm were all negative in that study, as were all non-size-fractionated PTFE filter cassette samples (3 µm pores).
Semelka CT 2021+	Viral transport media (VTM) on sedimentation plates from Anderson air samplers were pooled from stages 1 and 2 (filter sizes ≥5 µm) and stages 3–6 (filter sizes <5 µm) to separate large droplets from aerosols.	Viral particles in large respiratory droplets were recovered adjacent to the head from 2 of 26 patients (8%; droplet sizes ≥5 µm) who were closer to symptom onset (2 and 4 days). No aerosol-sized particles were detected in air samplers for masked or unmasked runs.
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 for 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 particle 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 ¹⁸ 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
Stern RA 2021 (a)	"Cascade samplers were located at floor height: (1) outside the entrance to a COVID-19 ward (CW1); (2) in personal protective equipment (PPE) donning room outside the entrance to another COVID-19 ward (CW2); (3) outside the entrance to the medical intensive care unit (ICU); (4) at a staff workstation in the emergency department (ED); and (5) at a nursing staff workstation of a ward not designated for the care of COVID-19 patients	In total 8 samples were positive: 2 for Fine (≤ 2.5 µm) particles and 3 each for Coarse (10.0–2.5 µm) and Large (> 10.0 µm)
Stern RA 2021 (b)	The study used custom-designed Harvard Micro-Environmental Cascade Impactors (Demokritou <i>et al.</i> , 2002) to collect simultaneous samples in three distinct size fractions: fine (≤2.5 µm aerodynamic diameter), coarse (2.5–10 µm), and large (≥10 µm)	In total 13 samples were positive: 3 for Fine (≤ 2.5 µm) particles and 7 for Coarse (10.0–2.5 µm) and 3 for Large (> 10.0 µm). The proportion of samples found positive was greatest for the symptomatic patient rooms (6/24 samples or 25%) with the highest viral concentration in these rooms (25 copies/m ³)
Zhang X 2022	Aerosols of 0.5 to 10 µm in diameter were collected using SASS 2300 Wetted Wall Cyclone Samplers (Research International, Inc. Monroe, WA, USA) operating at a flow rate of 325 liters per minute (L/min)	Aerosol particles of 0.5 to 10 µm in diameter were detected

surroundings of 23 hospitals and eight home-treated patients. Ma J 2020 *et al.* reported on an unventilated quarantine hotel toilet room from 26 samples taken and Moharir SC 2022 *et al.* sampled in hospital, the ICU and in patients' homes. Ong SWX 2021 *et al.* reported air samples from airborne-infection isolation rooms and a community isolation facility housing COVID-19 patients. Stern RA 2021 (b) *et al.* sampled 30 locations in a hospital and also a COVID-19 quarantine facility.

Liu Y & Ning Z 2020 *et al.* reported 4/13 public areas were RT-PCR positive; Zhang D 2020 *et al.* sampled the outdoor environment of three hospitals. Mallach G 2021 *et al.* sampled in rooms with COVID-19 positive patients and in long term care

homes. Similarly, Mouchtouri VA 2020 *et al.* sampled a hospital, a nursing home, and Long-Term Care Facility, but also included a ferryboat. Passos RG 2021 *et al.* reported environmental and hospital air sampling from May to August 2020.

Masoumbeigi H 2020 *et al.* sampled in a military hospital. Lednický JA 2020(b) *et al.* sampled in a respiratory infection evaluation area of a student healthcare centre and reported one positive sample with a CT of 39 (virus genome equivalent of 0.87 virus genomes L⁻¹ air).

Four studies reported on Exhaled Breath Condensate (EBC). Ma J 2020 *et al.* reported 14/52 EBC samples as RT-PCR

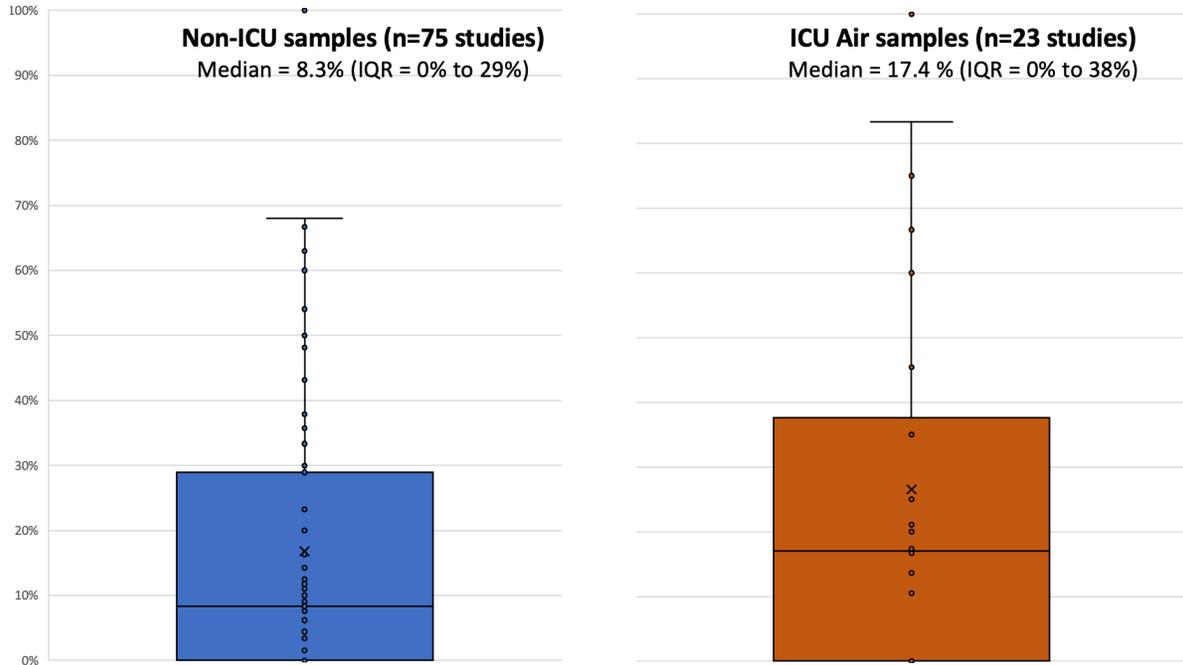


Figure 3. Proportion and distribution of SARs-CoV-2 RT-PCR positive Air samples in Hospitals and Intensive Care Unit (ICU) environments (n=80 studies).

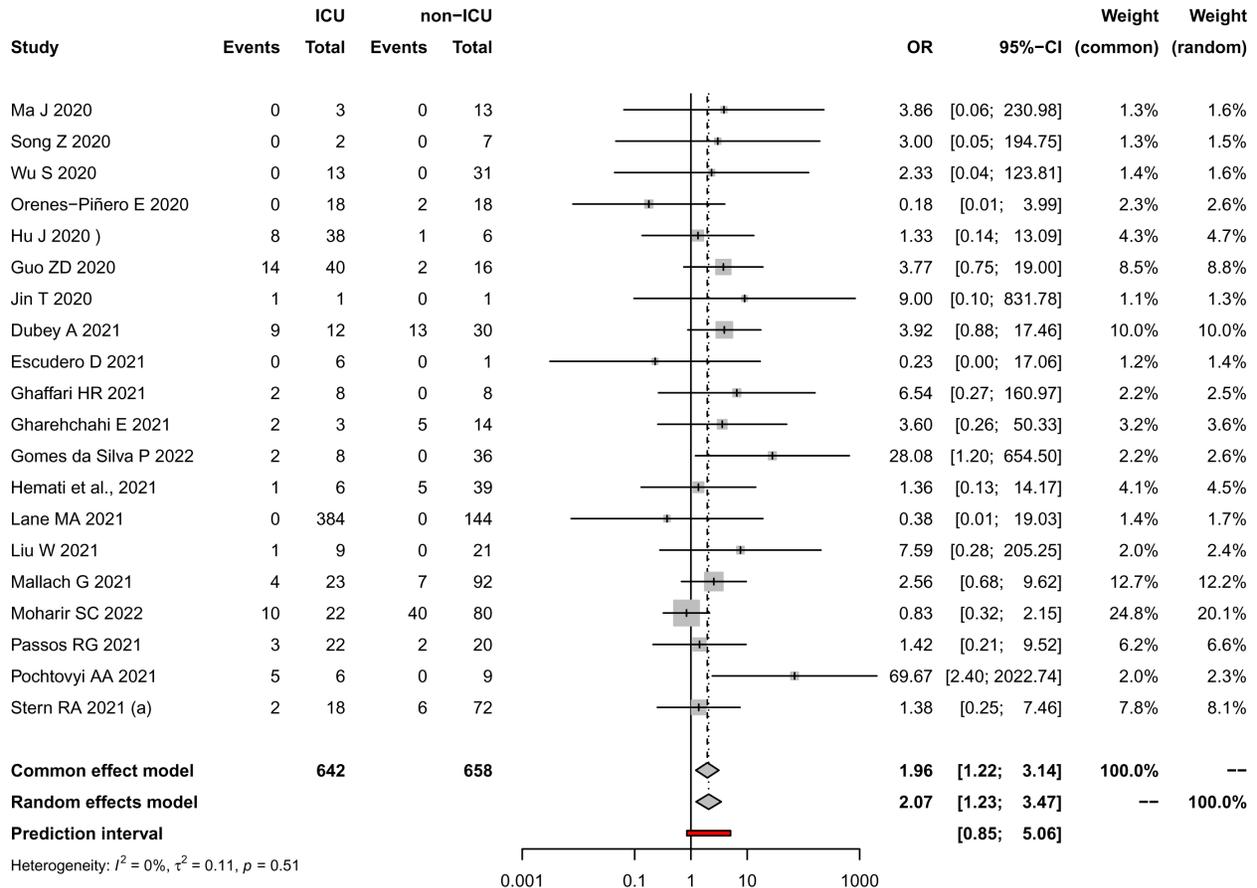


Figure 4. Forest plot showing the risk of presence of positive air samples of SARS-CoV-2 in hospitals.

positive and Feng B 2020 *et al.* reported 2/8 positive EBC samples. Zhou L 2020 *et al.* collected samples of exhaled breath of patients ready for discharge and air samples. Adenaiye OO 2021 *et al.*, sampled in a university campus and in the community and collected 30-minute exhaled breath samples while vocalizing into a Gesundheit-II sampler.

Community. Thirty-eight studies reported data in the community and did not sample in hospitals (see table of characteristics and [Figure 1](#)). Eight were done outdoors and/or in the community [Chirizzi D 2020, Kayalar O 2021, Kwon KS 2020, Linillos-Pradillo 2021, Pivato A 2021, Ramuta MD 2022, Setti L 2020, Dziedzinska R 2021]; five studies sampled buses [Di Carlo P 2020, Hoffman JS 2020, Luo K, 2020, Shen Y 2020 and Moreno T 2020 that also included sampling in subway trains). Four studies sampled in student rooms or university buildings [Adenaiye OO 2021: university campus and community; Pan J 2022: student rooms; Zhang X 2022: non clinical areas of University buildings and Lednicky JA 2020b: a student Healthcare centre.

Three studies sampled apartments/blocks of flats [Lin G 2020, Kang M 2020 and Nannu Shankar S 2021] and three nursing homes [De Man P 2020, Dumont-Leblond N 2021, Linde KJ 2022]. Two studies each sampled choir practices [Charlotte N 2020, and Hamner L 2020]; meat processing plants [de Rooij MMT 2021 and Günther T 2021]; restaurants [Li Y & Qian H 2020 and Lu J 2020]; quarantined households [Dohla M 202 and Horve PF 2021] that also included an isolation dormitory.

Seven studies sampled one setting each: a car [Lednicky JA 2021]; dental clinics [Bazzazpour S 2021]. an employee building [Li X 2022], a fitness centre [Li H 2021] a home residence [Wong JCC 2020] an indoor community setting [Conte M 2021] and a wastewater treatment plant [Gholipour S 2021].

Viral culture. Twenty-six studies attempted viral culture [Adenaiye OO 2021, Ang AX 2021, Ben-Shmuel 2020, Binder 2020, Coleman KK 2021, Dohla M 2020, Dumont-Leblond N 2020, Hu J 2020, Kotwa 2021, Lednicky JA 2020a, Lednicky JA 2020b, Lednicky 2021, Li X 2022, Linde KJ 2022, Lotta-Maria AH 2021, Mallach G 2021, Moharir SC 2022, Nannu Shankar S 2021, Nissen K 2020, Ong SWX 2021, Pan J 2022, Ruffina de Sousa 2022, Santarpia JL 2020a, Santarpia JL 2020b, Winslow R 2021, Zhou J 2020]. In 18 of these studies, infectious virus could not be isolated and cytopathic effects could not be observed [Ang AX 2021, Ben-Shmuel 2020, Binder 2020, Coleman KK 2021, Dohla M 2020, Dumont-Leblond N 2020, Hu J 2020, Kotwa 2021, Li X 2022, Lotta-Maria AH 2021, Mallach G 2021, Nissen K 2020, Ong SWX 2021, Pan J 2022, Ruffina de Sousa 2022, Santarpia JL 2020b, Winslow R 2021, and Zhou J 2020] (see [Table 6](#)).

Of the remaining eight studies, Adenaiye OO 2021 found culture-positive SARS-CoV-2 from two exhaled breath samples from participants while they were wearing face masks. None

of the fine aerosol samples collected when the participants were not wearing face masks tested positive on culture.

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 very low (approximately 340 virus genome equivalents). RT-PCR for SARS-CoV-2 RNA from the cell cultures were negative, but 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 United States. 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 competent replicating virus.

Lednicky JA 2021 reported positive culture in one out of four samples collected from inside a car driven by a SARS-CoV-2 positive patient. The passenger was sitting approximately 3 feet from the sampler.

Linde KJ 2022 reported positive cultures in one out of 10 air samples taken from the rooms of patients who were SARS-CoV-2 positive. The authors did not specify the distance from the patient from where the sample was collected.

Moharir SC 2022 reported positive cultures in one out of three air samples taken from the homes of patients who were SARS-CoV-2 positive. The authors did not specify the distance from the patient from where the sample was collected.

Nannu Shankar S 2021 reported positive culture in four out of 16 air samples taken from the home of a patient who was SARS-CoV-2 positive. However, the patient was co-infected with HAdV B3, which outgrew SARS-CoV-2 in Vero E6 cells. The authors stated that adenovirus B3 likely contributed to the respiratory symptoms experienced by the patient.

Santarpia JL 2020a reported 3/39 aerosol samples (particle size <1 µm) that cell culture resulted in increased SARS-CoV-2 RNA at very low levels. A virus-like particle 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. 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).

Table 6. Live culture results (n =26).

Study (n=64)	Setting	Method	Air Samples positive n/d for SARS-CoV-2 RNA	Live culture	Notes
Adenaiye OO 2021	University campus and community	COVID-19 cases series. Fomite (phone) swabs, and 30-minute exhaled breath samples	No mask coarse = 15/78 fine = 22/78 With mask coarse = 10/71 fine = 14/71	No mask coarse = 0/38 fine = 0/75 Mask coarse = 0/16 fine = 2/66	None of the fine-aerosol samples collected while not wearing face masks were culture-positive. Two exhaled breath samples and fine-aerosol samples collected from participants while wearing face masks were culture-positive.
Ang AX 2021	Hospital	Air and surface samples were collected from one isolation ward and two open-cohort wards housing laboratory-confirmed COVID-19 patients	13/27	0/27	High-flow rate air samplers, which provided higher sensitivity in detecting environmental SARS-CoV-2 in air when conducting surveillance in such indoor settings.
Ben-Shmuel 2020	hospital & quarantine hotel.	Surface and air sampling at two COVID-19 isolation units and in a quarantined hotel.	2/6 quarantine hotel 1/1	0/3	Relatively high CT values (>34) in the samples.
Binder 2020	Hospital	An observational case series of 20 patients hospitalized with coronavirus disease	3/195 samples from 3 patients	0/3 viable virus	
Coleman KK 2021	Hospital	Exhaled breath emitted by COVID-19 patients	13/22 participants 25/66 samples	0/13 participants 0/25 samples	Overall viral RNA loads were relatively low, they differed significantly between breathing, talking, and singing,
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	Infectious virus could not be isolated.	26/43 adults were positive for RT-PCR. 10/ 66 wastewater samples and 4/19 surface swab samples were RT- PCR positive
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	8/38 ICUs 1/6 CT rooms Samples from medical staff rest areas and corridors were all negative (denominator not clear)	All positive aerosol samples were negative	5/24 surface swabs in the ICU were PCR positive. After rigorous disinfection, no viral RNA was detected in a second batch sample from the same places. Positive rates for the mask samples were relatively high compared with the aerosol or surface samples. One mask from a critically ill patient was positive.
Kotwa, 2021	Hospital	Air and surfaces samples in rooms of COVID-19 patients	3/146	0/3	The three positive air samples were taken from 3 different rooms at 1 m from the patient

Study (n=64)	Setting	Method	Air Samples positive n/d for SARS-CoV-2 RNA	Live culture	Notes
Lednický JA 2020a	Hospital	Observational: air samples were collected, and virus culture attempted	4/4 air samples without a HEPA filter 0/2 samples using a HEPA filter	Virus-induced CPE was observed for 4/4 RNA-positive air samples.	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
Lednický 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	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
Lednický JA 2021	Car Journey	SARS-CoV-2 in a car driven by a COVID-19 patient. The PCIS sampler was attached to the sun-visor on the passenger side of the car, approximately 3 feet from the patient's face and with the intake port pointing toward the roof of the car, with the pump assembly placed on the front passenger seat.	4/5	1/4	The Cq of the culture positive sample was 29.65 days post-inoculation of Vero E6 cells. A Cq value of 12.46 was attained 3 days post-inoculation of the cells. The patient had minimal symptoms, and no viral concentration or infectiousness was established. The sampler was approximately 3 feet from the patient's face.
Li X 2022	Employee building	COVID-19 outbreak with two fast food employees infected, using environmental sampling, epidemiological tracing, viral RNA sequence, and surveillance method.	3/20 female washrooms n=2	0/3	
Linde KJ 2022	Nursing homes	Air samples were collected at three locations in the patient's room: 1) near the head of the patient within approximately 0.5 metres of the patient, 2) near the feet of bedridden patients, approximately 1.5 metres from the head or approximately 1.5 metres from mobile patients sitting in a chair, and 3) near the location often used by healthcare workers more than 2 metres away from the patient such as the sink, all positioned at 1.5m height.	Total: 94/213 Positive Oropharyngeal Swab (OPS) 93/184 Negative OPS 1/29 7/259 settling dust samples in three wards	1/10	All four air sampling techniques detected SARS-CoV-2 RNA and showed high rates of positivity in the rooms of patients with positive OPS CPE was observed in three OPS and one active air sample and confirmed by immunofluorescent staining. The active air sample from the CDC-NIOSH sampler (>4µm size fraction) had the lowest Ct of all environmental samples (29.5) and was from the room of the patient with the lowest OPS Ct-value (19.8). There was no information on the distance of the positive culture. However, the study reports that 'ultra-fine particles (<1µm), which can travel further, do not seem to be the key vehicle of SARS-CoV-2 transmission. The vast majority of settling dust and surface swab samples from common areas were negative, suggesting SARS-CoV-2 transmission is more a local phenomenon than widespread.'

Study (n=64)	Setting	Method	Air Samples positive n/d for SARS-CoV-2 RNA	Live culture	Notes
Lotta-Maria AH 2021	Hospital & Home	Air and surface samples from the surroundings of 23 hospitalized and eight home-treated COVID-19 patients	33/259 (12/29 air collections)	0/33	Seven different air collection methods were used.
Mallach G 2021	Hospital & Long term care home	Particulate air sampling in rooms with COVID-19 positive patients in hospital ward ICU rooms, long-term care homes and a correctional facility experiencing an outbreak.	ICU 4/23 Ward 7/92 LTC 3/15 Correctional facility 1/8	0/15	
Moharir SC 2022	Hospital & homes	Air, samples from different locations occupied by coronavirus disease (COVID-19) patients	Total 45/115 Hospital 40/80 (ICU 10/220) Closed rooms 5/17 homes 10/18	1/3 from the home setting	No details are provided for the culture results and no details on the viral concentrations beyond 'that had relatively lower Ct values'
Nannu Shankar S 2021	Apartments	Air and surfaces in bedrooms of two 20-year-old persons with symptomatic COVID-19 were sampled as self-isolating persons.	Volunteer A NIOSH 1/3 PTFE 0/3 Volunteer B NIOSH 4/6 PCIS 4/10	volunteer B Oct 2 4/8 Oct 6 0/8	Volunteer B was co-infected with HAAdV B3, which outgrew SARS-CoV-2 in our Vero E6 cells. Adenovirus B3 causes acute respiratory infections and likely contributed to the respiratory symptoms experienced by volunteer B.
Nissen K 2020	Hospital	Observational: surface swabs and fluid samples were collected, and experimental: virus culture was attempted.	7/19 filters 11 days later, 4/19 positive for both genes.	No significant CPE after three passages on Vero E6 cells	Ct values varied between 35.3 and 39.8 for the N and E genes. Virus culture was attempted: RNA was detected in sequential passages, but CPE was not observed.
Ong SWX 2021	Hospital & Community	Air samples from airborne-infection isolation rooms and a community isolation facility housing COVID-19 patients	6/12	0/6	Virus cultures were negative after 4 blind passages.
Pan J 2022	Student rooms	Surface swab samples and heating, ventilation, and air conditioning (HVAC) filters from 24 rooms occupied by students positive for COVID-19,	15/21 HVAC 4/6 bathroom exhaust grilles	Cultured those with a Ct value < 33, and none contained culturable virus.	No denominator for viral culture supplied
Ruffina de Sousa 2022	Hospital	Air samples from rooms occupied by COVID-19 patients in a major hospital.	patient rooms 9/22; adjoining anterooms 10/22	PFU recovery patient room 3/9; anteroom 8/10	Average Ct: patient rooms 38.3 and anterooms 38.3 Infectious viruses could not be isolated in Vero E6 cells from any environmental sample.

Study (n=64)	Setting	Method	Air Samples positive n/d for SARS-CoV-2 RNA	Live culture	Notes
Santarpia JL 2020a	Hospital	Observational: size-fractionated aerosol samples collected; experimental: virus culture was attempted.	6/6 patient rooms.	In 3 aerosol samples (<1 µm), cell culture resulted in increased viral RNA.	The presence of SARS-CoV-2 was observed via western blot for all but one of the samples (<1 µm, with statistically significant evidence of replication, by RT-PCR (Figure 2). The intact virus was observed via TEM in the submicron sample from Room. Viral replication of aerosol was observed in the 1 to 4 µm size but did not reach statistical significance.
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 were 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 metres 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).
Winslow R 2021	Hospital	Prospective observational study of 30 low SATS Covid-19 cases who received either supplemental oxygen, CPAP or HFNO	4/90	1/51 nasopharyngeal sample	One nasopharyngeal sample from an HFNO participant (E gene Ct 21.99) could demonstrate the presence of viable (infective) virus All other samples, including environmental samples, were negative. Samples were either positive or suspected positive for viral RNA and were cultured.
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

Table 7. Methodological issues in viral culture studies.

Study	Methodological
Adenaiye OO 2021	<ul style="list-style-type: none"> Logistical considerations required freezing samples between collection and culture, with the potential loss of infectiousness. Used a Gesundheit-II (G-II) exhaled breath sampler does not necessarily represent the real-world situation as samples are collected directly from patients, not the environment
Ang AX 2021	<ul style="list-style-type: none"> Sample collection and subsequent analysis were subject to the availability of the trained medical staff, consent of patients, and the capacity of the BSL-3 processing laboratory.
Ben-Shmuel 2020	<ul style="list-style-type: none"> There was a delay between the onset of symptoms and the actual sampling in patients' rooms. Therefore, at the time of sampling, these patients might not have shed viable virus,
Binder 2020	<ul style="list-style-type: none"> 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.
Coleman KK 2021	<ul style="list-style-type: none"> Used a Gesundheit-II (G-II) exhaled breath sampler (see Adenaiye 2021) Low viral load in the samples compared with those generally found in culturable clinical samples. Sampling methodology yielded viral RNA loads below 103.8 genome copies per sample,
Hu J	<ul style="list-style-type: none"> 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.
Kotwa, 2021	<ul style="list-style-type: none"> The median time between the onset of illness and air sampling was 11 days (IQR, 7–14); the time between the onset of illness and sampling date for all 3 PCR-positive air samples was 4 days. Air samples were excluded from the genomic sequence analyses due to poor quality sequences.
Lednický 2020a	<ul style="list-style-type: none"> it is not clear why plaque assays could not be performed due to a nationwide nonavailability of some critical media components in the US. Three serial 3-hr air samplings were performed. Over the 9 hours, patients likely would have moved about and may have been close 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 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. Nothing is presented about testing the air sampling isolates in susceptible animal models.
Lednický JA 2020b	<ul style="list-style-type: none"> 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). The PCR tests for SARS-CoV-2 vRNA from cell culture were negative, highlighting the essential requirement to test for other pathogens when general virus cytopathic effects are observed. Three respiratory viruses were identified: Influenza A H1N1, Influenza A H3N2, and Human coronavirus OC43
Lednický JA 2021	<ul style="list-style-type: none"> Two days after the diagnostic sample was obtained, the patient agreed to have the PCIS placed in her car (an older model Honda Accord) for the drive from the clinic to her home. The PCIS was attached to the sun-visor on the passenger side of the car, approximately 3 feet from the patient's face and with the intake port pointing toward the roof of the car, with the pump assembly placed on the front passenger seat. During the 15-min drive, the patient was not wearing a mask. Early CPE consistent with SARS-CoV-2 were observable by 3 days in cells inoculated with material collected onto PCIS filter D; by day 5, foci of infection were apparent for cells inoculated with material from filter D, with no signs of virus infection in cells inoculated with material collected by PCIS filters B, C, and E. For further confirmation, an aliquot (20 µL) of the virus collected 5 days post-inoculation of material from filter D was passaged in Vero E6 cells, wherein an rRT-PCR Cq value of 12.46 was attained 3 days post-inoculation of the cells.
Li X 2022	<ul style="list-style-type: none"> Two air samples collected on Dec. 20 and 21 from the female washroom without ventilation even after the disinfection were positive for SARS-CoV-2 with an estimated concentration level of 5640–7840 SARS-CoV-2 RNA copies m⁻³

Study	Methodological
Linde KJ 2022	<ul style="list-style-type: none"> • Among the 78 positive OPS, cyclone-based samples, impingement-based samples, surface swab samples, 44 had an RdRp Ct-value ≤ 35 and were investigated by virus culture. • CPE was observed in three OPS and one active air sample and confirmed by immunofluorescent staining. • The active air sample from the CDC-NIOSH sampler ($>4\mu\text{m}$ size fraction) had the lowest Ct-value of all environmental samples (29.5) and was from the room of the patient with the lowest OPS Ct-value (19.82). • If the virus-induced cytopathic effect was observed, immunofluorescent detection of nucleocapsid proteins was performed to confirm the presence of SARS-CoV-2 • Limited information on the virus culture was reported
Lotta-Maria AH 2021	<ul style="list-style-type: none"> • Seven different air collection methods were used. • Only conducted environmental sampling at a single time point.
Mallach G 2021	<ul style="list-style-type: none"> • were careful to always sample two or more meters from COVID-19 patients, to ensure detection of the virus only at distances traditionally considered to be consistent with the airborne transmission. • The mean Ct values were just over and under 34 for the N and E proteins, respectively. The Ct value was <34 for the N protein in only one room, and <34 for the E protein in eight rooms • No direct sampling of patients was performed to determine their infectiousness, and we did not have access to patient history • Almost all hospitalized patients were admitted at least five days after symptom onset, when they are less likely to be shedding infectious virus,
Moharir SC 2022	<ul style="list-style-type: none"> • Many of the air samples from hospitals and closed room experiments showed PCR signal for one of the SARS-CoV-2 genes or had very high Ct values. • No details on culture results or on samples beyond the three from the home setting
Nannu Shankar S 2021	<ul style="list-style-type: none"> • "Virus-induced CPE were observed in Vero E6 cells inoculated with air and surface samples collected from volunteer B's room within 4 days of their inoculation. Since the Cq value was high (>34) when nucleic acids extracted from the cell growth media of the cell cultures were tested by RT-qPCR for SARS-CoV-2. • The study authors suspected an additional respiratory virus was present, as previously observed in Lednicky <i>et al.</i>, 2020b and Pan 2017) • Volunteer B was co-infected with HAdV B3, which outgrew SARS-CoV-2 in our Vero E6 cells. Adenovirus B3 causes acute respiratory infections and likely contributed to the respiratory symptoms experienced by volunteer B. • There was an Inconsistent use of samplers and no measurements on aerosol size distribution.
Ong SWX 2021	<ul style="list-style-type: none"> • Selected patients early in their illness course and with a lower Ct value because they hypothesized this would maximize the possibility of successfully isolating viable viruses. • Most patients had only mild disease, • Sampling was conducted in a naturally ventilated community isolation facility, and airborne-infection isolation hospital rooms (designed to limit transmission of airborne infections)
Pan J 2022	<ul style="list-style-type: none"> • Viral load estimates were made by extrapolating information on the amount of RNA found on the rooms' HVAC filters. • Results suggest that SARS-CoV-2 decays within the amount of time between the student vacating the room and sampling in this study (ranging from 6 h to 4 days).
Ruffina de Sousa 2022	<ul style="list-style-type: none"> • Patients were entering their second week of the disease, and SARS-CoV-2 titers in the upper respiratory tract tend to peak in the first week of disease - Median days since onset (IQR) 11.5 (7–14) • No CPE was observed • Average Ct in the patient rooms 38.3 and anterooms 38.3 was too high for viable viral culture

Study	Methodological
Santarpia JL 2020a and b	<ul style="list-style-type: none"> • For Santarpia 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 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). • Neither plaque assay nor serial passage was attempted in the study. The statistical inferences are very difficult to interpret in Figure 1 when you look at 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 is much higher than what most labs use and accept and a lot of background "noise" as a result • It is likely an equation was 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
Winslow R 2021	<ul style="list-style-type: none"> • The authors remark they found no significant differences with the environmental variables. • There was no relationship between days unwell at the time of sampling, or nasopharyngeal Ct values between those who did and did not have viral RNA in air samples. • Participants in our study were on average in their second week of illness when admitted to the hospital (mean 9-days) and when sampled (mean 12-days). • Plated specimens in the presence of antibiotics and antimycotics and after incubation of 5 days plaques were subjected to RT-PCR for agent identification. A good, well-reported descriptive study. Very low evidence of environmental contamination and only one NP specimen showed infectivity. • No evidence that CPAP or any of the other procedures raised the risk of infectiousness. The report shows a breakdown of Ct by gene and comments on CPE, with confirmatory PCR. Shows correlation between symptoms and Ct and air samples in the range of 35–40 Ct. • Samples with at least one log increase in copy numbers for the E gene (reduced Ct values relative to the original samples) after 5–7 days propagation in cells compared with the starting value were considered positive by viral culture.
Zhou J 2020	<ul style="list-style-type: none"> • No indication of 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.

Discussion

We identified 128 primary observational studies that showed RT-PCR SARS-CoV-2 RNA can be detected in airborne samples in a variety of settings both indoors and outdoors. Several 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/or the location and variable distance of the sampling methods. Control sampling for concomitant bacterial or fungal organisms (which can also produce cytopathic effects on cell monolayers) was not generally done, which would serve as useful controls. In one study which looked for multiple bacteria, fungi, and viruses,

including SARS-CoV-2, using qPCR assays, they found much higher burdens of nucleic acids from multiple species of commonly encountered pathogenic and non-pathogenic bacteria (e.g., coagulase negative staphylococci and enterococcus and some Gram-negative bacilli), *Candida* species and Herpes simplex virus and on all sampling days in comparison to the small quantities of SARS-CoV-2 RNA in their airborne samples¹⁵. These findings suggest that the presence of bio-aerosolized DNA or RNA from multiple microbes in hospitals is commonplace, and none of these commonly-encountered organisms are considered to be transmitted by the airborne route.

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 tuberculosis¹⁶.’ 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 viruses¹⁷. For coronaviruses including SARS-CoV-1 and MERS-CoV, previous review evidence supporting the airborne route of transmission is weak¹⁸; The majority of the studies included in our systematic review and reported in the tables, do not find evidence to support the airborne transmission route. 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 with low Ct values [Mponponsoo K 2020]; and Wong SCY *et al.* reported that none of 120 contacts of a patient with initially undetected COVID-19 subsequently became infectious.

Strengths and limitations

There is a current dearth of well-conducted high-quality studies addressing airborne transmission. To our knowledge, this is the most comprehensive review assessing airborne transmission of SARS-CoV-2. We extensively searched the literature, and we accounted for the reporting quality of the included studies, including the methods used for air sampling and viral culture. However, we recognize several limitations. The findings of our review are limited by the low-quality of the included studies that lack standardised protocols, methods, reporting and outcomes. The small sample sizes, the absence of study protocols and the lack of replication further limit any firm conclusions to be drawn from the findings. Sporadic isolation of viral RNA may be due to problems with sampling techniques. Furthermore, while our search was comprehensive, we may have missed some studies. The lack of standardised reporting means it can be difficult to find essential study details about the methods and the results.

Implications for research

Evidence from the referenced systematic reviews we found noted the need to improve the quality of the primary studies. Anderson *et al.* reported the need for further data collection under differing temperature and humidity conditions¹⁹. Carducci *et al.* considered no studies had sufficient confirmatory evidence, and airborne transmission remains hypothesis-driven²⁰. Schünemann *et al.* noted direct studies in COVID-19 are limited and poorly reported²¹, and Mousavi *et al.* noted the need for rigorous and feasible lines of research in the area of air filtration and recirculation in healthcare facilities²².

Future studies are warranted to verify findings before definitive conclusions can be reached about modes of transmission and including important knowledge regarding the minimal infectious dose for a specific mode of transmission. Because of the heterogeneity of the settings, the case-mix limitations, the timing between symptom onset and sampling, the sampling techniques used, the lack of 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 standardisation requires attention as it limits the development of general recommendations for the sampling of airborne viruses²³.

One essential question is whether observed epidemiologic associations are causal^{24,25}. Establishing transmission modes requires integrated epidemiological and mechanistic approaches to narrow uncertainty⁹. 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 should ensure that there is evidence of exposure to a transmissible agent. 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. Identifying those circumstances that promote transmission using all relevant evidence that would be more likely to promote viral transmission is important, as well as for identifying interventions. Any study based on epidemiological associations regarding infectious agents should ideally have confirmation from whole genome sequencing. Sequencing has repeatedly shown that outbreaks initially thought to share a single origin were, in fact, the product of multiple independent infection events²⁶.

It is worthy to note that when conducting environmental sampling only a small fraction of the detectable nucleic acids is necessarily incorporated into virus particles, and not all the particles are intact and infectious. It can also take variable numbers of infectious virions to initiate an infection, with this “minimal infectious dose” varying depending upon many factors including the disease agent, route of infection, the host, host age, underlying health conditions, and host immune status. Even a relatively straightforward measurement like

particles-to-PFU varies widely among different viruses²⁷. Of special importance is data from a recent human challenge trial²⁸ where an intranasal dose of 10 TCID₅₀ (~7 PFU) virus yielded 53% attack rates. Given that one PFU corresponds to ~160,000 genome copies in human clinical specimens²⁹ one can then estimate that an exposure to >1 million genome copies might be required to yield a ~50% chance of infection. Given the high Ct values detected in the majority of air samples, and the poorly designed and reported virological assays, further research and standardisation of the protocols used to measure genome copies and assay for virus are required in clarifying whether air samples of SARS-CoV-2 are truly infectious.

We found that air samples in the same hospital were more likely to be positive in ICU environments than in the non-ICU. These results are homogenous. However, this observation should be interpreted with caution as the lack of information on the individual demographics of the patients (e.g., symptom onset, underlying illness and degree of immunocompromise) and lack of standardisation across the studies limits the complete interpretation of the result. Detection of SARS-CoV-2 RNA in the air cannot confirm transmission, since only infectious virions can cause disease, but it can be a useful tool for surveillance.

Because of the widespread misunderstanding over the role of PCR positivity in assigning transmission causation, we have proposed a framework for reporting studies that assess causality that helps strengthen the methods used for conducting and reporting respiratory virus transmission research³⁰. The reporting of viral RNA concentrations was heterogeneous as were the sampling methods.

Our proposed framework requires serial viral culture, genome sequencing and evidence that the source was sufficiently contaminated (low Ct) with infectious material (cultivable virus) to transmit infection to another human. Availability of all such evidence provides a high standard of proof of transmission³⁰.

In some studies, the setting fitted within the definition of transmission in a close contact setting. For example, in Lednický JA 2021 and Linde KJ 2022, the distances between the index patients and the exposure participants (from which positive cultures were reported) were within 3 feet and 6 feet respectively.

None of the included studies definitively demonstrated that replication-competent SARS-CoV-2 can be recovered in the air, which offers the most robust evidence of transmissibility³¹. 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 confirming the exclusion of other pathogens or contaminants with sequence confirmation.

General virus-induced CPE were observed in Lednický JA 2020b however, RT-PCR tests for SARS-CoV-2 were negative while three other respiratory viruses were identified: Influenza A H1N1, Influenza A H3N2, and human coronavirus OC43.³². Similarly, Nannu Shankar S 2021 reported positive

culture in 4/16 air samples from a patient's home. However, the patient was co-infected with HAdV B3, which outgrew SARS-CoV-2 in Vero E6 cells. Both studies demonstrate the importance of testing cultured samples for other viruses.

In further versions of this review, we plan to focus solely on those studies that attempted serial viral culture, given its vital role for establishing transmission causality. This is similar to the methods we used to assess the transmission of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) from pre and asymptomatic infected individuals¹⁴. By reviewing only the high-quality studies we were able to provide probable evidence of SARS-CoV-2 transmission from presymptomatic and asymptomatic individuals. This update required writing to authors to clarify methods and obtain missing information this is beyond the scope of this current update. We have published a protocol outlining the additional methods³³.

Conclusion

SARS-COV-2 RNA can be detected by RT-PCR in the air in a variety of settings. The lack of definitive consistently recoverable viral culture samples of SARS-CoV-2 prevents firm conclusions to be drawn about the relative contribution of airborne transmission of this virus. Although airborne transmission of SARS-CoV-2 cannot be ruled out, particularly in certain situational settings, further research is required to investigate the plausibility of such transmission. The current evidence is low quality, and there is a 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

Previous version of this data were stored on Figshare, <https://doi.org/10.6084/m9.figshare.14248055.v2¹⁰>.

The extended data for this version is available at the Open Science Framework

SARS-CoV-2 and the role of airborne transmission: a systematic review. <https://doi.org/10.17605/OSF.IO/PE876>

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.v2¹⁰>.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license \(CC-BY 4.0\)](https://creativecommons.org/licenses/by/4.0/).

Acknowledgements

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Open Peer Review

Current Peer Review Status:    

Version 3

Reviewer Report 25 May 2023

<https://doi.org/10.5256/f1000research.139143.r172710>

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 **Jennifer Grant** 

Faculty of Medicine, The University of British Columbia, Vancouver, British Columbia, Canada

Thank you for this interesting study. A couple of comments:

1. It would be nice to know in which way you modified the QUADAS 2 tool if it is possible to put that in an annex or supplementary materials, for reproducibility.
2. Greater clarity between the difference of detection of virus and transmissibility would be helpful to orient the reader in the introduction. In fact, this review is not a study of *transmission* but rather the presence of viral nucleic acid which *may* indicate the potential for transmission.
3. I will quibble with your statement (description of table 4) that Ct Values <25 are associated with risk of transmission since this is based on patient samples, not environmental samples. Given the complexities of understanding transmission, this is an extrapolation that cannot be demonstrated with any confidence. In fact, based on your presented data, with positive SARS-CoV-2 cultures only in very specific circumstances (car, exhaled breath samples), there appears to be no CT threshold associated with infectivity based on viral culture. Since you report no data on onward transmission in the studies presented, there is not data on infectivity based on detected infections either.
4. It would be helpful for the reader to document, in the results section, those viral cultures that definitively detected SARS-CoV-2 growth rather than simply detecting cytopathic effect given the potential for other viruses to cause the same CPE without being SARS-CoV-2.
5. While it is true that having a high concentration of virus that is replication competent is necessary to cause infection, it has not been shown to be sufficient. Therefore it would not stand as a "high standard of proof of transmission," as stated in your discussion. These criteria could stand as a high standard for the potential for transmission, but, at the end of the day, the only proof of transmission is transmission itself which could be shown either by experimental infection or through animal studies (e.g. ferret studies in influenza), or as you correctly state through serial passage of infectious virus through cell-lines.

6. Please include a discussion of ferret experiments in the transmission of influenza in your discussion of airborne transmission of other respiratory infections as this is the most natural argument for the potential for SARS-CoV-2.

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?

Yes

Is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are the conclusions drawn adequately supported by the results presented in the review?

Yes

If this is a Living Systematic Review, is the 'living' method appropriate and is the search schedule clearly defined and justified? ('Living Systematic Review' or a variation of this term should be included in the title.)

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Patient Quality and Safety, infectious diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 05 October 2021

<https://doi.org/10.5256/f1000research.77065.r93516>

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Maosheng Yao 

College of Environmental Sciences and Engineering, Peking University, Beijing, China

I just read their responses to my comments. First, a thank you to the authors for taking their valuable time to respond. Unfortunately, I found none of my critiques have been adequately answered. Thus, I feel that I do not need to further comment this paper. It seems to me that the

authors are reluctant to revise their paper as commented. For most of my comments, the authors said they are out of scope of their paper, although they state that their paper is a “systematic” review. If they state many things, despite vital to the review, are out of the scope of the paper, they should at least change their paper title to something: a mini review, not a systematic review. Overall, I am not satisfied with any responses to my comments.

It is unusual to see that they refused to change their conclusion even the WHO has officially recognized the aerosol transmission of COVID-19. As another reviewer pointed out they performed a biased review. It would be very harmful if misleading statements or documents are endorsed by influential authors from prestigious institutions on a public domain especially in face of the disastrous pandemic. I think if the authors were right with their position of the COVID-19 transmission, the pandemic would already have been well under control since disinfection and 1-meter distancing were all in place across the world since March 2020. However, the reality is that the pandemic is still ravaging the world.

It is important that the paper be revised according to the comments raised by many online authors and reviewers in order to avoid causing further confusion and misleading the public on combating the pandemic. This is what I, as a reviewer, can write and can do regarding this matter.

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?

Yes

Is the statistical analysis and its interpretation appropriate?

Yes

Are the conclusions drawn adequately supported by the results presented in the review?

Yes

If this is a Living Systematic Review, is the ‘living’ method appropriate and is the search schedule clearly defined and justified? (‘Living Systematic Review’ or a variation of this term should be included in the title.)

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I obtained a PhD in conducting bioaerosol related studies from Rutgers University; and did postdoc training at Yale in the same field. I am currently a Professor from Peking University, and has been working in bioaerosol field for about 20 years. My expertise ranges from bioaerosol sampling and detection to air pollution health effects and particulate matter toxicity.

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.

Reviewer Report 16 September 2021

<https://doi.org/10.5256/f1000research.77065.r93514>

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David R. Tomlinson 

University Hospitals Plymouth NHS Trust, Plymouth, UK

Dear Professor Heneghan and team,

Thank you for responding to my submitted comments following my review of version 1 'SARS-CoV-2 and the role of airborne transmission: a systematic review'. I hope that my responses herein to your comments and manuscript changes are of use to you and your team.

1. Re: my request for a revision to the stated definition of an aerosol.

Thank you for revising the stated definition.

Note: This additional section has been pasted into your response, but I believe this relates to comments made by reviewer 3 and was in error?

"There are varied definitions of aerosols in the published literature. An aerosol is defined as a collection of particles (liquid or solid) with varying aerodynamic diameters, suspended in the air (gas) for a prolonged period of time. The size of the particles and the distance they may travel is highly variable and depends on many factors, (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2843947/>; https://apps.who.int/iris/bitstream/handle/10665/112656/9789241507134_eng.pdf;jsessionid=41AA684FB64571CE8D)"

Consider to add this reference Xie X, Li Y, Chwang AT, Ho PL, Seto WH. How far droplets can move in indoor environments--revisiting the Wells evaporation-falling curve. Indoor Air. 2007 Jun;17(3):211-25. doi: 10.1111/j.1600-0668.2007.00469.x. PMID: 17542834

2. Re: my request for you to address evidence of plagiarism in paragraph 2 of the introduction (55% match to text from Kutter *et al.* (2018), your original reference 4, now reference 7).

Thank you for making changes. However, the first sentence of the new text you have provided in your response does not match that of version 2 manuscript:

Your response to my review: *"Transmission via droplets and aerosols in specific settings or situations may potentiate the spread of some viruses in humans, resulting in disease outbreaks that are difficult to manage."*

Version 2 manuscript: "Transmission via **droplet nuclei** and aerosols in specific settings or situations may potentiate the spread of some viruses in humans, resulting in disease outbreaks that are difficult to manage."

I would be grateful if you could clarify which of these is the correct and final version, please. Thank you.

3. Re: my request to provide objective methodological descriptors of factors employed to determine the selection of included studies (i.e., concerns over terms including 'adequately', 'sufficient' and 'clearly defined') and assess their quality.

Thank you for providing a response to this together with my point 6: a request for 'objectively defined descriptions of 'Quality of included studies' in 'your table 3'.

You have provided a far clearer description of the methods used here and I am pleased to see your qualifying statement regarding the use of the 'QUADAS 2 risk of bias tool' towards **quality reporting** [my term], since QUADAS 2 was never intended to be used to assess study quality. Instead, it was devised towards 'more transparent rating of bias and applicability of primary diagnostic accuracy studies'¹. However, again there are differences in the text between the 'tracked changes' version 2 manuscript submitted to the F1000 editorial office, and the text in your response to my comments, as follows:

Your response: "*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 low⁹.*

Version 2 manuscript: "We assessed quality using a modified QUADAS 2 risk of bias tool¹¹. We simplified the tool **because** the included studies were not designed as primary diagnostic accuracy studies, and **there is a lack of high-quality data in published transmission studies.**"

I would be grateful if you could clarify which of these is the correct and final version, please. Thank you.

In your extended description of the methods here, you have provided useful insights into how study quality was assessed, thank you. However, this new description of 'quality reporting' [my term] scoring - i.e., low / moderate / high - remains importantly imprecise. For example, under point (ii) you state:

"Methods – did the study authors sufficiently describe the methods used to enable replication of the study? E.g. methods used for diagnosing SARS-CoV-2 in patients, procedure used for air sampling, time-point for sampling, number of samples per site, cycle thresholds, culture methods, airflow/ventilation settings, humidity".

From this total of eight factors, how many of these would constitute your scoring of low vs moderate vs high quality? Similarly, under each point you have not clarified how each of the multiple stated 'quality factors' [my term] was used to achieve your final 'quality reporting'. Therefore, for your newly described study methods to be considered reproducible, these points require further clarification please. In so doing, this will also help provide assurance to readers

that study quality assessment processes were without important bias. Thank you.

You later state:

"The risk of bias for each domain was rated "low", "moderate" or "high" depending on the adequacy of reporting. One reviewer (CJH) assessed the risk of bias while a second author (EAS) independently verified the risk of bias."

I believe these are typographical errors, since you had modified the QUADAS 2 risk of bias tool to assess **study quality**, not bias. I would be grateful if you would correct these sentences accordingly, please. Thank you.

One further important methodological point arises here from review of some of the new text provided in which you state:

"(v) Follow-up - was the pattern and number of air samples sufficient to demonstrate airborne transmission - e.g. repeat sampling, serial sampling?"

This newly clarified list of 'quality indicators' suggests that you consider a **single** air sample demonstrating live SARS-CoV-2 to be insufficient evidence towards airborne transmission of SARS-CoV-2. Whatever the argument regarding the purely circumstantial level of evidence afforded by air sampling studies (Kutter *et al*, your reference 7), please could you provide justification for this requirement for repeated findings of this nature? Thank you.

4. Re: my concerns over inappropriately narrow study selection criteria towards assessing the evidence base for SARS-CoV-2 airborne transmission (e.g., exclusion of laboratory studies of SARS-CoV-2 aerosol viability, animal models of transmission within controlled environmental conditions).

My concern over the difference in methodology comparing the 'Protocol for a living evidence review (Version 3: 1 December 2020)' and this present manuscript (i.e. the former stated under 'Study inclusion and exclusion': "Eligible studies should include sampling for the detection of SARS-CoV-2 in the population or the environment on any potential mode of transmission..." whereas the latter states: "We included field studies that included airborne sampling for SARS-CoV-2 in the population under study or the environment."). The former methodology permits the inclusion of studies that have formed the historical basis for investigations into the transmission routes of many human pathogens (e.g. outbreak reports within households, hospitals and/or aircraft), including the demonstration that measles is transmitted via the airborne route, whereas the latter does not).

In your response, you have not explained why you changed the protocol from that originally outlined in your online protocol and nor have you provided clarification of this methodological change in the final manuscript. I would be grateful if you could include this point in your final manuscript, please. Thank you.

Thank you for including this sentence in version 2, study limitations:

"We excluded study designs/settings that attempted to detect SARS-CoV-2 via other methods apart from

air sampling, e.g., virus stability, outbreak reports, aircraft outbreaks, non-pharmaceutical intervention, experimental infection, air tracer studies and computational modelling/simulation."

However, from a 'scientific' perspective this statement alone is insufficient: given that you have chosen to exclude methods which represent the foundational approach to historical studies towards understanding routes of pathogen transmission, you must provide a valid scientific argument for excluding each of these experimental types. Moreover, you must provide an explanation of how any resulting narrow methodological search strategy remains a valid indicator of 'real world' SARS-CoV-2 transmission characteristics, particularly since Kutter *et al.* (your reference 7) correctly state that air sampling studies – your current restricted study inclusion criterion – only provide circumstantial evidence towards airborne transmission.

Furthermore, if animal transmission studies remain excluded from your final manuscript, you must explain the scientific validity of this decision, particularly considering that transmission in animal models represents one of the 10 foundational methods employed by the Centers for Disease Control and Prevention (CDC) in its Influenza Risk Assessment Tool (IRAT) – as you know, an 'assessment tool developed by the CDC and external influenza experts that assesses the potential pandemic risk posed by influenza A viruses that currently circulate in animals but not in humans.' (source, <https://www.cdc.gov/flu/pandemic-resources/national-strategy/risk-assessment.htm>)

In addressing my concerns over the exclusion of airborne viability experiments conducted in March 2020, you state:

"Laboratory studies such as the one quoted provide insights into the stability of the virus in airborne suspensions but provide no insights into whether there exist ordinary biological mechanisms capable of generating such high-titer aerosols in the first place. The fact that one can put humans into orbit, doesn't mean it is an easily achieved or common task. It simply says humans can survive in orbit."

Thank you for clarifying your views. However, considering this explanation, your newly included reference 4 – used to better define the meaning of the term 'aerosol' – states: *"Aerosols are produced when an air current moves across the surface of a film of liquid, generating small particles at the air-liquid interface. The particle size is inversely related to the velocity of air."* (Annex A, first sentence of body text, page 37/156).

May I respectfully remind you and your co-authors that during every respiratory cycle – an 'ordinary biological mechanism' – in mammals, air currents move across the surface of a film of liquid (respiratory fluid) lining the endothelium of the respiratory tract. It is self-evident that humans create aerosols during tidal breathing. Furthermore, in experimental studies of human subjects during influenza infection dating back as far as 1966, sufficient quantities of influenza virus aerosols have been demonstrated to be released during coughing, to result in levels of environmental air contamination necessary to sustain transmission via the airborne route (Couch RB *et al.*, (1966)².

Also, a 2013 investigation of children and adults during respiratory virus infection², referenced by World Health Organisation COVID-19 Infection Prevention and Control Scientific Brief authors, July 2020 in their discussion of possible airborne transmission of SARS-CoV-2 ([https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-](https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for)

infection-prevention-precautions), stated:

"The simultaneous detection of viral RNA in small, airborne-sized (<4.7 mm) and large, droplet-sized (≥ 4.7 mm) particles produced on breathing and coughing by children and adults with symptomatic respiratory infections was observed in this study. Viruses detected included hRV, Influenza A virus, Parainfluenza 1 and 3 viruses and RSV"². Using the PubMed search criteria 'cough' AND 'aerosol' demonstrates 1,392 published manuscripts since 1967-2020 inclusive.

I hope there is no further need for me to reference this extensive literature for you and your co-authors to accept that abundant aerosol release occurs during 'ordinary biological' activities including breathing, speaking, singing, coughing, and sneezing. Accordingly, it is a biological inevitability that any pathogen contained within fluid lining the respiratory tract and which demonstrates aerosol viability, has the potential for airborne transmission when the time course of its airborne viability exceeds a critical threshold. Considering that the aerosol viability experiments conducted by Van Doremalen *et al.*³ demonstrated SARS-CoV-2 survival for hours, clinically important airborne / aerosol transmission of SARS-CoV-2 is inevitable, according to these universally applicable physical laws. It will therefore probably come as no surprise to learn of more recent data from humans with COVID-19, demonstrating that: "fine aerosols [$<5\mu\text{m}$ diameter] constituted 85% of the SARS-CoV-2 viral load emitted during singing, talking, or breathing."³ In short, just like SARS Coronavirus [1], SARS-CoV-2 is airborne.

Now considering your review comment:

"The inclusion of laboratory studies was not a part of our protocol but could be included as a part of a separate review but is outside the scope of our study."

This present study protocol is simply not fit for purpose when it comes to addressing the stated objective in your online Web appendix 1 Protocol for a living evidence review (Version 3: 1 December 2020): *"to provide a rapid summary and evaluation of **relevant data** [my emphasis] on transmission of SARS-CoV-2..."*. You have provided no scientifically valid justification for such a limited study protocol in your responses, and as such your study protocol must be changed to include **all** studies of relevance, i.e., as stated in my original response, virus stability, outbreak reports, aircraft outbreaks, non-pharmaceutical intervention, experimental infection, air tracer studies and computational modelling/simulation. Thank you.

5. Re: my concern over your questioning the occurrence of airborne transmission of SARS-CoV-2 according to first principles of similarity in biological properties of closely related viruses (i.e., my statement that SARS Coronavirus was proven to be airborne (2003), so SARS-CoV-2 will be airborne too).

The principle of 'concordant 'biological properties of closely related viruses can be found in the 'WHO Ebola 2014 IPC guideline', which is why I included this reference in my comments. In responding to this point, you cast doubt on the proven airborne transmission of SARS Coronavirus. I was hoping that I had perhaps misinterpreted your reply, but this statement in the discussion of your present manuscript is concerning:

"For coronaviruses, previous review evidence supporting the airborne route of transmission is weak (reference cited: Herfst et al 2017)".

Searching this quoted reference for evidence in support of this statement reveals the following (first paragraph, page 27):

'Furthermore, for some microorganisms, for example, for coronaviruses, epidemiological or experimental evidence that transmission of the pathogens via the airborne route is successful or even contributes importantly to epidemic or pandemic spread of the agent remains weak.' - Astonishingly, Herfst *et al.* (2017) provide no reference in support of this assertion, so this statement lacks scientific validity.

I am surprised that you were not aware of this fact, since the Herfst *et al.* manuscript was your choice of reference to support your assertion against airborne transmission of SARS Coronavirus. Furthermore, given some of your fellow contributing authors' expert standing in the infectious diseases / infection prevention and control community, they must be aware of the WHO declaration that 'SARS is an airborne virus' (https://www.who.int/health-topics/severe-acute-respiratory-syndrome#tab=tab_1). Accordingly, please would you explain: (1) why you have denied airborne transmission of SARS Coronavirus, given the international consensus on the existence and clinical importance of this transmission route? (2) Why did you try to support your statement by using a manuscript whose authors failed to provide a reference to support their statement: 'epidemiological or experimental evidence that transmission of the pathogens [i.e., coronavirus] via the airborne route is successful or even contributes importantly to epidemic or pandemic spread of the agent remains weak'? Thank you.

6. Re: my comment, '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.'

You respond as follows:

"We already have a published protocol that has been used to conduct our series of systematic reviews of studies investigating transmission dynamics of COVID-19."

As described in detail above, you have importantly deviated from this original protocol without providing suitable justification on scientific grounds. Furthermore, the objectives of this present systematic review (i.e., 'to provide a rapid summary and evaluation of relevant data on transmission of SARS-CoV-2', from the Web Appendix 1, Protocol for a living evidence review Version 3: 1 December 2020) cannot be met using such restricted study inclusion criteria.

You continue:

"However, our research is ongoing, the quality of the evidence and methods have changed over time and we make necessary adjustments to improve the robustness of the evidence as more studies (and evidence) become available (and examined)."

I am glad that your team's research is ongoing, since this meets your stated aim: *'there is a need to continuously and systematically conduct reviews of publicly available studies with the latest knowledge from publications to inform WHO recommendations using the most up-to-date information.'* (Web

Appendix 1, Protocol for a living evidence review (Version 3: 1 December 2020). However, it is surprising that despite previously undertaking an updated literature review every 2 weeks between 1st February and 20th December 2020 for version 1, the time-period for included literature in this present version 2, has not been extended beyond December 2020. In short, why not? Why would any research team with a stated protocol aim *'to provide a rapid summary and evaluation of relevant data on transmission of SARS-CoV-2'*, fail to update their manuscript in the 6 months since its first release?

Furthermore, I am sure there would be huge benefit in extending the date range for your search, not least in view of your repeated criticism of methods and stating:

"Standardized guidelines for conducting and reporting research on airborne transmission are needed", the February 2021 manuscript of Borges JT *et al.* is likely to be of great interest to you 'SARS-CoV-2: a systematic review of indoor air sampling for virus detection'⁴. But this is just one of very many published studies which demonstrate conclusively that SARS-CoV-2 transmission occurs via the airborne route. Indeed, taking a simple mathematical approach to assess the probability of aerosol inhalation versus large droplet-based SARS-CoV-2 transmission between humans, led me to the figure of 56 million to one in favour of aerosol inhalation. I'd be keen to know whether you agree with my logic (<https://twitter.com/DRTomlinsonEP/status/1436214995239456769?s=20>).

Thank you again for responding to my comments. I hope you find my responses useful towards your stated aim: *"to provide a rapid summary and evaluation of relevant data on transmission of SARS-CoV-2"*.

References

1. Whiting PF, Rutjes AW, Westwood ME, Mallett S, et al.: QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med.* 2011; **155** (8): 529-36 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Couch RB, Cate TR, Douglas RG, Gerone PJ, et al.: Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. *Bacteriol Rev.* 1966; **30** (3): 517-29 [PubMed Abstract](#) | [Publisher Full Text](#)
3. Coleman K, Wen Tay D, Tan K, Xiang Ong S, et al.: Viral Load of SARS-CoV-2 in Respiratory Aerosols Emitted by COVID-19 Patients while Breathing, Talking, and Singing. *medRxiv.* 2021. [Publisher Full Text](#)
4. Borges JT, Nakada LYK, Maniero MG, Guimarães JR: SARS-CoV-2: a systematic review of indoor air sampling for virus detection. *Environ Sci Pollut Res Int.* 2021; **28** (30): 40460-40473 [PubMed Abstract](#) | [Publisher Full Text](#)

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?

Yes

Is the statistical analysis and its interpretation appropriate?

Yes

Are the conclusions drawn adequately supported by the results presented in the review?

Yes

If this is a Living Systematic Review, is the 'living' method appropriate and is the search schedule clearly defined and justified? ('Living Systematic Review' or a variation of this term should be included in the title.)

Yes

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.

Version 1

Reviewer Report 05 May 2021

<https://doi.org/10.5256/f1000research.55319.r82052>

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Maosheng Yao 

College of Environmental Sciences and Engineering, Peking University, Beijing, China

Review for Heneghan *et al.* (2021), SARS-CoV-2 and the role of airborne transmission: a systematic review, F1000Research 2021, 10:232 .

First, I have to declare that this review is provided solely based on scientific evidence and reasoning without any discipline preferences or conflicting interests. Despite not being exhaustive, I have to greatly applaud the efforts of all the authors for developing this review, especially the compiling of relevant COVID-19 aerosol related articles. However, to me this review serves as a perfect piece for evidence compiled for probable airborne transmission of COVID-19 if the biased discussion and preferences of content inclusion are eliminated/avoided. Thus, I feel this review, if to be indexed, needs to completely change the tone, and better suited for an article with a title like: Evidences for probable aerosol transmission of COVID-19: a systematic review. For its current form, there are many problematic issues with this review, and the discussion is also biased.

Major comments are listed below:

1. This review is commissioned and supported by the WHO (World Health Organization) which has clearly stated in March 2020 that COVID-19 is not airborne. An effort supported by the WHO is hardly believed to use to overturn its own statements or harm its authority. Thus, the authors should provide documents that can demonstrate such potential conflicting interests can be sufficiently cleared. Otherwise, I can only say that this review just serves as a proxy of the WHO for its statements with a biased science provided.

2. The aerosol definition was not effectively or properly communicated. For example, in the Introduction, they state that “aerosol particle ranges from 0.001 μm to over 100 μm ”. Are they sure that there are 10 cm (100 mm) aerosol particles in the air? In case they mistyped the unit, e.g., 100 μm should be used. Experimental data in many studies (they did not cite) show that major fraction of exhaled particles during breathing or speaking in controlled and real world scenarios are smaller than 5 μm , and sometimes they peak at 1 μm . Most recently, US CDC states that surface contact transmission of COVID-19 is minimal (the authors should certainly update the review). Therefore, how do the authors explain the COVID-19 transmission between people? They might attribute the droplet transmission. But, airborne droplet is an aerosol by definition. The review did not provide any data for droplet transmission. Instead, they have provided a lot of studies that have detected SARS-CoV-2 in aerosol particles.

3. This review lacks a significant amount of discussion of aerosol physics (very important) in terms with aerosol transmission of COVID-19. For example, a lot of studies they provided used a filter to sample air, and it is known that filtration itself can cause desiccation which affects the integrity of biological cells/particles over a prolonged time period. Accordingly, it partially attributed to low recovery of viable SARS-CoV-2 virus. For impaction or liquid based studies, the sampling velocity could also damage the integrity of the viruses, e.g., the BioSampler has an impact velocity of up to 300 m/s by calculation, which would somehow damage the virus. Besides, when the viruses are released into the air, they would be rapidly diluted and transported away given any ventilation of either natural or mechanical nature is present. Thus, the airborne viral concentration level is time, ventilation, and space dependent. The aging of the virus in the air also affect its viability. In addition, the in vitro viability tests with cells can not be directly translated back to the infection of human cells inside the body where the overall physiological environment is different, and more favorable for viral replication. So far, no studies have demonstrated that those in vitro tested non-infectious viruses cannot infect humans. Human inhalation takes place at a rate of about 12 L/min, which is relatively gentle in terms of sampling stress on the virus. Therefore, their argument that

lack of recovery of viable virus prevents a firm conclusion of airborne transmission of COVID-19 is not supported by their reasoning and existing data. Instead, many outbreaks or infections are difficult to explain without referring to airborne/aerosol transmission.

4. In their review, I do not know how they could define a low quality study. They stated that all 67 primary studies are of low quality. However, these studies are published by peer-reviewed journals including those premier ones. If they are of low quality, how they could pass the rigorous screening of these journals? On the other hand, this review did not state which are high quality studies and they did not provide them either. I think they should at least provide high quality data to support their conclusion. It seems they used "low quality data" to produce "high quality" conclusions.

5. For bioaerosol studies, there are no unified or standardized methods or procedures. Different studies have different purposes, different circumstances, different set of sampling tools (the efficiencies could vary greatly; in terms of sampling biological agents higher physical efficiency usually results in higher damage). So, different sampling tools have very different efficiencies. Because viral level is greatly diluted in the air, higher volume or longer sampling time is required to enrich enough viral particles for detection. Most of the studies they complied used RT-PCR for quantification. Depending on the detection kit used, the efficiencies could also vary greatly. Generally, the detection limits of RT-PCR are higher, and accordingly those samples with low viral level would be tested negative. If a more sensitive method such as digital PCR (1 copy per uL) was used, higher percentage of positive samples would be reported.

6. Clearly the review did not discuss how any ventilation would affect the airborne viral levels. Air is constantly moving in not enclosed environments. Thus, air sampling is very time sensitive. Besides, emission of viral particles by the patients might not be continuous. Airborne detection of SARS-CoV-2 is highly time dependent in ventilated environments.

7. For airborne transmission, increasing physical distance significantly reduces the viral levels depending on the indoor building ceiling height and ventilation status. Human inhalation of the airborne virus is a comparably gentle sampling (causing less damage), and the respiratory tract provides a better incubation environment for SARS-CoV-2. Thus, mechanical air sampling together with in vitro viability tests cannot confirm the true non-infectiveness of airborne SARS-CoV-2 given the results are negative. In addition to shared space, shared time in an indoor space might be also important for airborne transmission to occur. Shared time would allow the virus not to age for too long in the air before inhaled. All of this should be discussed in the review.

8. The tables take up most of the review, however high quality discussion is lacking. It is often observed that the statements in the review lack references.

Minor comments

1. Technical presentation of the data are not good. To me, all the tables prepared are like a laundry list of items without in-depth discussion or elaboration. Often, some important information present in certain studies they cited are not included in the table or in the discussion. For example, Ma *et al.* (2020)¹ found COVID-19 patients emit millions of SARS-CoV-2 during just breathing, implying great potential of aerosol transmission of the diseases. Breathing produces fine aerosol particles. However, they did not elaborate on this. Nonetheless, viable SARS-CoV-2

was indeed recovered from hospital air. But, the review did not provide direct data against the airborne transmission.

2. There are many grammar mistakes throughout the manuscript as pointed out by other reviewers.

3. The debate or discrepancy might primarily arise from different understanding and definition of aerosol, droplet or airborne transmission from different communities. Aerosol concentration is higher in close ranges, while it is substantially diluted with increasing physical distance, like injecting a drop of ink into a sea. The dose and viability of the virus also play important roles in terms of causing an infection. This should be discussed in a more neutral tone in the review.

I strongly encourage the authors to include aerosol scientists to provide a comprehensive and correct guidance/review that the WHO can use to save millions of lives. Time and resources for certain regions are running out & actions need to be taken immediately.

I have provided some references for the authors to further read the details of relevant topics I have discussed in the report:

Greenhalgh, Trisha, Jose L. Jimenez, Kimberly A. Prather, Zeynep Tufekci, David Fisman, and Robert Schooley. [Ten scientific reasons in support of airborne transmission of SARS-CoV-2](#). *The Lancet* 397, no. 10285 (2021): 1603-1605.

Morawska, Lidia, Julian W. Tang, William Bahnfleth, Philomena M. Bluysen, Atze Boerstra, Giorgio Buonanno, Junji Cao *et al.* [How can airborne transmission of COVID-19 indoors be minimised?](#). *Environment international* 142 (2020): 105832.

Yao, Maosheng, Lu Zhang, Jianxin Ma, and Lian Zhou. [On airborne transmission and control of SARS-Cov-2](#). *Science of The Total Environment* 731 (2020): 139178.

Wilson, Nick, Stephen Corbett, and Euan Tovey. [Airborne transmission of covid-19](#). *BMJ* 370 (2020).

Morawska, Lidia, and Donald K. Milton. [It is time to address airborne transmission of coronavirus disease 2019 \(COVID-19\)](#). *Clinical Infectious Diseases* 71, no. 9 (2020): 2311-2313.

Prather, Kimberly A., Linsey C. Marr, Robert T. Schooley, Melissa A. McDiarmid, Mary E. Wilson, and Donald K. Milton. [Airborne transmission of SARS-CoV-2](#). *Science* 370, no. 6514 (2020): 303-304.

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1. Ma J, Qi X, Chen H, Li X, et al.: COVID-19 patients in earlier stages exhaled millions of SARS-CoV-2 per hour. *Clin Infect Dis*. 2020. [PubMed Abstract](#) | [Publisher Full Text](#)

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

No

Are sufficient details of the methods and analysis provided to allow replication by others?

No

Is the statistical analysis and its interpretation appropriate?

Partly

Are the conclusions drawn adequately supported by the results presented in the review?

No

If this is a Living Systematic Review, is the 'living' method appropriate and is the search schedule clearly defined and justified? ('Living Systematic Review' or a variation of this term should be included in the title.)

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I obtained a PhD in conducting bioaerosol related studies from Rutgers University; and did postdoc training at Yale in the same field. I am currently a Professor from Peking University, and has been working in bioaerosol field for about 20 years. My expertise ranges from bioaerosol sampling and detection to air pollution health effects and particulate matter toxicity.

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.

Author Response 25 Aug 2021

Carl Carl

Peer Reviewer #3

First, I have to declare that this review is provided solely based on scientific evidence and reasoning without any discipline preferences or conflicting interests. Despite not being exhaustive, I have to greatly applaud the efforts of all the authors for developing this review, especially the compiling of relevant COVID-19 aerosol related articles. However, to me this review serves as a perfect piece for evidence compiled for probable airborne transmission of COVID-19 if the biased discussion and preferences of content inclusion are eliminated/avoided. Thus, I feel this review, if to be indexed, needs to completely change the tone, and better suited for an article with a title like: Evidences for probable aerosol transmission of COVID-19: a systematic review. For its current form, there are many problematic issues with this review, and the discussion is also biased.

Revised: Thanks. We have made several revisions to the original submission and we believe the quality of the manuscript is much improved. We do not agree with the reviewer that the title must be changed based on our rigorous review of the evidence presented within the papers we assessed.

Major comments are listed below:

1. This review is commissioned and supported by the WHO (World Health Organization)

which has clearly stated in March 2020 that COVID-19 is not airborne. An effort supported by the WHO is hardly believed to use to overturn its own statements or harm its authority. Thus, the authors should provide documents that can demonstrate such potential conflicting interests can be sufficiently cleared. Otherwise, I can only say that this review just serves as a proxy of the WHO for its statements with a biased science provided.

Response: The guidance by the WHO on modes of transmission of SARS-COV-2 is constantly evolving and has done so since March 2020. The reviewer has made assumptions which are simply untrue and inaccurate. Such documents do not exist as WHO had no influence in any of our reviews, the process included for the reviews or the interpretation of the results. This standard of proof is not required by editors for submitted manuscripts (CH has been an Editor-in-Chief of a BMJ journal, IJO has been a research editor and JMC has also been an Editor-in-Chief of a Journal). A peer reviewer who has submitted previous research would know the requirements for submission according to the ICMJE criteria. The insinuation of overt bias by this reviewer is unfounded and the statement should be retracted.

2. The aerosol definition was not effectively or properly communicated. For example, in the Introduction, they state that "aerosol particle ranges from 0.001 μm to over 100 mm". Are they sure that there are 10 cm (100 mm) aerosol particles in the air? In case they mistyped the unit, e.g., 100 μm should be used. Experimental data in many studies (they did not cite) show that major fraction of exhaled particles during breathing or speaking in controlled and real world scenarios are smaller than 5 μm , and sometimes they peak at 1 μm . Most recently, US CDC states that surface contact transmission of COVID-19 is minimal (the authors should certainly update the review). Therefore, how do the authors explain the COVID-19 transmission between people? They might attribute the droplet transmission. But, airborne droplet is an aerosol by definition. The review did not provide any data for droplet transmission. Instead, they have provided a lot of studies that have detected SARS-CoV-2 in aerosol particles.

Response: We have redefined aerosol (see the response to peer reviewer #1 above).

3. This review lacks a significant amount of discussion of aerosol physics (very important) in terms with aerosol transmission of COVID-19. For example, a lot of studies they provided used a filter to sample air, and it is known that filtration itself can cause desiccation which affects the integrity of biological cells/particles over a prolonged time period. Accordingly, it partially attributed to low recovery of viable SARS-CoV-2 virus. For impaction or liquid based studies, the sampling velocity could also damage the integrity of the viruses, e.g., the BioSampler has an impact velocity of up to 300 m/s by calculation, which would somehow damage the virus. Besides, when the viruses are released into the air, they would be rapidly diluted and transported away given any ventilation of either natural or mechanical nature is present. Thus, the airborne viral concentration level is time, ventilation, and space dependent. The aging of the virus in the air also affect its viability. In addition, the in vitro viability tests with cells can not be directly translated back to the infection of human cells inside the body where the overall physiological environment is different, and more favorable for viral replication. So far, no studies have demonstrated that those in vitro tested non-infectious viruses cannot infect humans. Human inhalation takes place at a rate

of about 12 L/min, which is relatively gentle in terms of sampling stress on the virus. Therefore, their argument that lack of recovery of viable virus prevents a firm conclusion of airborne transmission of COVID-19 is not supported by their reasoning and existing data. Instead, many outbreaks or infections are difficult to explain without referring to airborne/aerosol transmission.

Response:

We have shown the bewildering array of samplers used in the studies. The reviewer raises a good point, but it is a subject matter beyond the scope of our systematic review and our protocol. We might suggest the reviewer and others in the field could contribute to establish an international standard for air capture and hope that all primary studies conform to that standard which we will be quite happy to report in future reviews.

The review by Verreault and colleagues (Methods for sampling of airborne viruses. *Microbiol Mol Biol Rev.* 2008;72(3):413-444. doi:[10.1128/MMBR.00002-08](https://doi.org/10.1128/MMBR.00002-08)) sets out some of the basic principles in improving the methods in this area and has been included as a reference in the revised manuscript.

'Many types of samplers have been used over the years, including liquid impingers, solid impactors, filters, electrostatic precipitators, and many others. The efficiencies of these samplers depend on a variety of environmental and methodological factors that can affect the integrity of the virus structure. The aerodynamic size distribution of the aerosol also has a direct effect on sampler efficiency. Viral aerosols can be studied under controlled laboratory conditions, using biological or nonbiological tracers and surrogate viruses, which are also discussed in this review. Lastly, general recommendations are made regarding future studies on the sampling of airborne viruses.'

4. In their review, I do not know how they could define a low quality study. They stated that all 67 primary studies are of low quality. However, these studies are published by peer-reviewed journals including those premier ones. If they are of low quality, how they could pass the rigorous screening of these journals? On the other hand, this review did not state which are high quality studies and they did not provide them either. I think they should at least provide high quality data to support their conclusion. It seems they used "low quality data" to produce "high quality" conclusions.

Response: We have expanded the methods section describing how we assessed study quality (see the response to peer reviewer #1 above).

5. For bioaerosol studies, there are no unified or standardized methods or procedures. Different studies have different purposes, different circumstances, different set of sampling tools (the efficiencies could vary greatly; in terms of sampling biological agents higher physical efficiency usually results in higher damage). So, different sampling tools have very different efficiencies. Because viral level is greatly diluted in the air, higher volume or longer sampling time is required to enrich enough viral particles for detection. Most of the studies they complied used RT-PCR for quantification. Depending on the detection kit used, the

efficiencies could also vary greatly. Generally, the detection limits of RT-PCR are higher, and accordingly those samples with low viral level would be tested negative. If a more sensitive method such as digital PCR (1 copy per uL) was used, higher percentage of positive samples would be reported.

See response to comment 3.

Response: We agree that the methods can be improved and made more sensitive. This should be the focus of future work but is not an area that we are able to address.

6. Clearly the review did not discuss how any ventilation would affect the airborne viral levels. Air is constantly moving in not enclosed environments. Thus, air sampling is very time sensitive. Besides, emission of viral particles by the patients might not be continuous. Airborne detection of SARS-CoV-2 is highly time dependent in ventilated environments.

Response: We agree and ventilation issues were considered to be beyond the scope of work outlined for this systematic review. It would be a very interesting topic for a future systematic review.

7. For airborne transmission, increasing physical distance significantly reduces the viral levels depending on the indoor building ceiling height and ventilation status. Human inhalation of the airborne virus is a comparably gentle sampling (causing less damage), and the respiratory tract provides a better incubation environment for SARS-CoV-2. Thus, mechanical air sampling together with in vitro viability tests cannot confirm the true non-infectiveness of airborne SARS-CoV-2 given the results are negative. In addition to shared space, shared time in an indoor space might be also important for airborne transmission to occur. Shared time would allow the virus not to age for too long in the air before inhaled. All of this should be discussed in the review.

Response: Thanks for the comments. We appreciate the points raised but these mechanistic hypotheses are beyond the scope and intent of our systematic review. A separate review of mechanistic modes of transmission would be of value in the future.

8. The tables take up most of the review, however high quality discussion is lacking. It is often observed that the statements in the review lack references.

Response: The tables report the evidence, we can comment but it's up to the readers to consider our conclusions.

Minor comments

1. Technical presentation of the data are not good. To me, all the tables prepared are like a laundry list of items without in-depth discussion or elaboration. Often, some important information present in certain studies they cited are not included in the table or in the discussion. For example, Ma *et al.* (2020)¹ found COVID-19 patients emit millions of SARS-CoV-2 during just breathing, implying great potential of aerosol transmission of the diseases. Breathing produces fine aerosol particles. However, they did not elaborate on this. Nonetheless, viable SARS-CoV-2 was indeed recovered from hospital air. But, the review did

not provide direct data against the airborne transmission.

Response: We have attempted to lay out the information in the tables in line with previous reviews. We have also revisited the information to ensure the information presented is relevant.

Ma and colleagues suggested COVID-19 patients recruited in Beijing exhaled millions of SARS-CoV-2 **RNA copies** into the air per hour and that exhaled breath emission may play an important role in the COVID-19 transmission. The Ct values of the breath and air samples, respectively (35.54 ± 3.14 and 38.40) are extremely high and not compatible with infectious virus based on other studies that have correlated infectious virus and Ct values (Jefferson T, Spencer EA, Brassey J, Heneghan C. Viral cultures for COVID-19 infectious potential assessment - a systematic review. Clin Infect Dis. 2020 Dec 3:ciaa1764. doi: 10.1093/cid/ciaa1764. Epub ahead of print. PMID: 33270107; PMCID: PMC779932). The mere presence of RNA copies based on PCR sampling does not imply infectiousness and the reviewer comments suggesting millions of SARS-CoV-2 intact viruses are emitted during breathing and capable of causing infection does not have evidence to support the statement from what we have been able to find.

Response: Meaningful inference can only be drawn from solid evidence.

2. There are many grammar mistakes throughout the manuscript as pointed out by other reviewers.

Response: Thanks. We have checked for grammatical errors and typos.

3. The debate or discrepancy might primarily arise from different understanding and definition of aerosol, droplet or airborne transmission from different communities. Aerosol concentration is higher in close ranges, while it is substantially diluted with increasing physical distance, like injecting a drop of ink into a sea. The dose and viability of the virus also play important roles in terms of causing an infection. This should be discussed in a more neutral tone in the review.

Response: We have already commented on the confusing nature of the definitions of aerosol, droplet and airborne transmission.

I strongly encourage the authors to include aerosol scientists to provide a comprehensive and correct guidance/review that the WHO can use to save millions of lives. Time and resources for certain regions are running out & actions need to be taken immediately.

Response: We have substantial expertise and experience within our team (see the response to peer reviewer #2 above).

Of note, in undertaking peer review the ICMJE states: 'Reviewers should declare their relationships and activities that might bias their evaluation of a manuscript and recuse themselves from the peer-review process if a conflict exists.' Intellectual conflicts would introduce such bias to peer review assessments. And according to

COPE's Ethical Guidelines for Peer Reviewers, COPE also highlights that the professional responsibility underpinning the peer review requires the necessary expertise to assess the manuscript and can provide an unbiased assessment.

We do not engage in emotive statements regarding the reviewers' opinions. We ensure our comments are evidence-based, unbiased and reflect the best available evidence. The reviewer should note that we do not speak for WHO and two co-authors of this review are currently collaborating with aerosol scientists to look for high-quality evidence relating to the mechanism(s) of transmission.

Competing Interests: No competing interests were disclosed.

Reviewer Report 22 April 2021

<https://doi.org/10.5256/f1000research.55319.r82064>

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Nancy H. L. Leung 

WHO Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, Hong Kong

In this systematic review, Heneghan *et al.* attempted to summarise the literature on the role of airborne transmission for SARS-CoV-2, with a focus of air sampling studies or epidemiologic studies that may evaluate the aerosol mode of transmission. They described that all primary research studies selected were low quality, probably attributing to the lack of standard methods, sampling sizes and reporting items. They concluded that SARS-CoV-2 was intermittently detected in the air, but the lack of recoverable viral culture samples prevents conclusions over airborne transmission.

I applaud the authors' attempt in summarising the current literature. I also agree the results of the selected studies are heterogeneous, and that currently there is very minimal number of studies that demonstrated infectious virus recovered in the air. This review would have been very useful as an evidence base for future discussion on the importance of aerosol transmission; however, the lack of objective and systematic evaluation of the methodology used in the selected studies precludes such usefulness. My major concern is that the review set out with the assumption that "the quality of transmission studies is known to be low" (reference #9 was also irrelevantly cited as described further below), although one of the main purposes of this review would be to evaluate the quality of evidence of each study. The benchmarks used to evaluate whether "Analysis & reporting outcomes (are) appropriate" (Table 3) were not described, but which were critical to evaluate the quality of each study. The assumption of air sampling studies were of poor quality in general can be felt along the manuscript, especially in Table 6 with a very large paragraph of criticism on Santarpia 2020, but personally I think a lot of evaluation on this

particular study was misguided due to insufficient understanding of the methodology used (as described further below). In some instances, studies were being described as poorly done without explanation of why the authors think so. I think including authors who have working knowledge in the field of air sampling studies and/or epidemiologic transmission studies would help to improve this review greatly.

There are also quite a number of missing information in the Tables, truncated sentences and typos, which requires a thorough re-read and check.

Please also find my specific comments below:

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

- For this definition of airborne (aerosol) transmission, emerging discussion has suggested aerosol transmission occurs in both short- and long-range (see my review¹).

A collection of particles (liquid or solid) ranging in size from 0.001 μm to over 100 μm suspended in a gas defines an aerosol.

- This is the classic definition of aerosols from the discipline of occupational hygiene, but other disciplines with a specific focus on bioaerosols with the origin of infectious pathogens may differ based on different aspects of transmission¹.

Method

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.

- These classification of study design are ambiguous; for example, what was the intervention being studied in interventional studies? Shouldn't clinical reports, outbreak reports and case-control studies be classified as observational studies? Were retrospective observational studies included, and on the other hand randomised trials would always be prospective? Overall, if these study designs are merely summaries of studies identified from systematic search based on well-defined search terms and are not being used for inclusion/ exclusion of studies, I would suggest to move these descriptions to the Results section instead, and only keep those that are relevant to study selection in the Method section (e.g. "Studies should include sampling for the detection of SARs-CoV-2.")

Studies should include sampling for the detection of SARs-CoV-2.

- Do you mean "air sampling for the detection of SARS-CoV-2"?

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 m³, viral culture methods and results, size of air particles (when reported) and proportion in the sample.

- For "copies per m³", do you mean "copies per m³ air sampled"? For "proportion in the sample", what is the numerator and the denominator - e.g., NIOSH sampler is commonly used in air sampling study, and for each time of collection the same volume of air are segregated into 3 size-fractions, would this be counted as 1 or 3 air samples? Alternatively, some studies will use multiple samplers in the same collection (e.g. being placed at different locations in the patient room), would this be considered as multiple samples or one sample

(run)? A clear definition is needed to allow comparison between studies, and should also be clearly described in Table 1.

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 low⁹.

- As listed in Table 3, one of the criteria was "Analysis & reporting outcomes appropriate". What were the benchmarks that were being used to be evaluated against about whether the analysis or the reporting outcomes are appropriate or not?
- The use of reference #9 here is inappropriate as it refers to transmission during the symptomatic or asymptomatic phase, with no mention of any modes of transmission.
- Truncated sentence ("after tool,").

Results

Limitations of the sampling methods and the poor-quality reporting make it difficult to discriminate between airborne or droplet nuclei transmission.

- Is "airborne" a typo here?

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.

- Comparative refers to comparison between what?

Overall the reporting was heterogeneous.

- Do you mean the methods or the results in the reported studies were heterogeneous?

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).

- Should "outdoors" be considered as healthcare settings? Similarly in the paragraphs follow about outdoors and community, actually I would think the distinction lies in outdoors vs. indoors, and within indoor higher-risk (e.g. healthcare settings/ households with confirmed cases) vs lower-risk (restaurants/ public transport etc)

(142 positives out of 1,403 samples: average 10.1%).

- Please refer to my above comments on the numerator/ denominator for the proportion of samples - can the proportions from different studies (which may refer to different things) be combined? What does this 10.1% represent/ how to interpret?

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 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).

- I suggest to move this paragraph to a new section, as these evidence refers to whether a transmission event has occurred (i.e. whether someone is infected), which is a different outcome measure from recovering virus in the air.

Discussion

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.

- Please elaborate what (1) the weaknesses and (2) the lack of validated methods are referring to

Past attempts to detect infectious particles have proved difficult: aerosols are dilute and culturing fine particles is problematic.

- Why is culturing fine particles (as opposed to coarse particles?) problematic, apart from being diluted?

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 tuberculosis¹¹.'

- There was clear evidence in terms of observed transmission event via the aerosol route for measles, chickenpox and rhinovirus¹.

For coronaviruses, previous evidence supporting the airborne route of transmission is weak¹³.

- Please clarify that this review was published before the COVID-19 pandemic.

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.

- How do air sampling studies conducted outdoors (as opposed to indoors), or the lack thereof, suggest the studies are not well-conducted - should the studies be evaluated based on methodological robustness instead (e.g. sampling duration, attempt to recover infectious virus, etc.)?

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.

- exposure to an infectious agent?

No airborne study to date definitively demonstrates SARS-CoV-2 is of an infectious nature, which offers the most robust evidence of transmissibility²².

- Do you mean "SARS-CoV-2 _recovered in the air_"? Do you mean "evidence of aerosol transmission" instead of "transmissibility" (please note the difference between "transmissibility" and "modes of transmission"¹)? I'm not sure whether identifying infectious virus in the air is the most robust evidence of aerosol route, as whether aerosol transmission can take place also depends on the susceptibility of the infected person to the aerosol route, and I would think a demonstration of transmission event takes place via the aerosol route would be a stronger evidence.

Table 1

- Binder 2020: *which decreases to approximately 40% efficiency for aerosols ~80 µm in diameter - --> typo.*
- Charlotte N 2020: please describe the lack of ventilation as described in Charlotte *et al.*
- Horve PF 2020: *14/56 s ---> typo.*
- Li & Qian 2020: missing notes.
- Lu J 2020: please describe the study scenarios/ findings that were relevant to why the study was selected (e.g. airflow consistent with transmission).
- Mponponso 2020: why was this study selected? the type of high-risk behaviour/ procedure has not been described in the study?
- Bahl P 2020: please be aware of plagiarism and rephrase.
- Ji B 2020: Missing main results.
- Singhai S 2020: Missing main results and key conclusions.
- Hussain A 2020: Missing key conclusions.
- Correia G 2020: Missing main results.

Figure 1

- Full-text articles excluded because no transmission outcome studied ---> What does

'transmission outcome' refer to here? From my understanding, 'transmission outcome' refers to whether an infection is initiated in an exposed person; but most of the air sampling studies included in this review did not demonstrate such 'transmission outcome'

Figure 2

- For "Was follow up sufficient", referring to Table 2, shouldn't the 83.6% mostly be "Not Applicable" instead of "No/Unclear"?

Table 4

- please check to see the use of unit symbol is consistent in the Table (uM vs. um; copies/L vs. /L, copies/m³ vs. copies m³, m² vs. m³, m³ vs. m3).
- Chirizz D 2020: please kindly indicate which size ranges were reported.
- Horve PF 2020: truncated sentence.
- Liu Y & Ning Z 2020: typo ("rang-").
- Zhou J 2020: 101 copies per how much air?

Table 5

- Feng B 2020: truncated sentence.

Figure 4

- Similar to my comments about numerator/ denominator above, were the proportions reported between studies directly comparable?

Table 6

- Binder 2020: a Ct of <20 would be considered as high viral load that may be sufficient to be culturable?
- Hu J: missing publication year.

Lednický 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. ---> I think Lednický et al. meant that due to lack of components which make up the culture media (for cultivating the cells to be infected), plaque assay (which involves the use of cells to be infected) cannot be performed.*

Santarpia JL 2020a:

- *For Santarpia 2020 (a) we could only find a preprint publication. ---> it is now published in Scientific Reports.*
- *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. ---> Please elaborate on this statement - how to explain the increased viral RNA presence if it is not because of viral replication?*
- *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. ---> In contrast to this statement, referring to Figure 2 in Santarpia JL 2020a, mock (negative control) samples have already been included. Anti-SARS nucleocapsid protein (SARS-CoV N) antibody were used in Western blot which is specific to SARS-CoV-2 virions. A significant test has been done for viral load between day 1 vs. day 5/6, and significant increase in viral load would be suggestive of viral replication between these days.*

- *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. ---> The NIOSH samplers (commonly used in air sampling studies) were used in this study, which size-fractionated the sampled air.*
- *No plaques were reported to have been detected and no serial passage on subculture was reported. ---> Plaque assay nor serial passage was attempted in the study.*
- *Statistical inferences are very difficult to interpret in Figure 1 based on the error bars. ---> Student's t test was done to compare viral load between day 1 vs. day 5/6.*
- *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. ---> Refers to above comment on the use of NIOSH samplers in this study.*
- *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. ---> Virus in the sample was being measured directly to obtain Ct values, which was then translated to viral load based on standard curve (i. e. a serial dilution of virus of different concentration) from a known quantity of SARS-CoV-2 virus.*
- *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. ---> The significant increase in viral RNA from day 1 to day 5/6 would be suggestive of viral replication.*

Table 7

- Santarpia JL 2020b: *Partial evidence of virus replication from one air sample.* ---> typo.

References

1. Leung NHL: Transmissibility and transmission of respiratory viruses. *Nat Rev Microbiol.* 2021. [PubMed Abstract](#) | [Publisher Full Text](#)

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?

Partly

Is the statistical analysis and its interpretation appropriate?

Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?

Partly

If this is a Living Systematic Review, is the 'living' method appropriate and is the search

schedule clearly defined and justified? ('Living Systematic Review' or a variation of this term should be included in the title.)

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious disease epidemiology; aerosol transmission; modes of transmission; respiratory viruses; air sampling studies; field studies

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 25 Aug 2021

Carl Carl

Peer reviewer 2

1. In this systematic review, Heneghan *et al.* attempted to summarise the literature on the role of airborne transmission for SARS-CoV-2, with a focus of air sampling studies or epidemiologic studies that may evaluate the aerosol mode of transmission. They described that all primary research studies selected were low quality, probably attributing to the lack of standard methods, sampling sizes and reporting items. They concluded that SARS-CoV-2 was intermittently detected in the air, but the lack of recoverable viral culture samples prevents conclusions over airborne transmission.

Response:

Thank you. Our team includes an expert virologist, a vaccinologist and an infectious disease specialist and epidemiologists. We have expanded our methods section to show how we assessed the quality of included studies (see response above).

2. I applaud the authors' attempt in summarising the current literature. I also agree the results of the selected studies are heterogeneous, and that currently there is very minimal number of studies that demonstrated infectious virus recovered in the air. This review would have been very useful as an evidence base for future discussion on the importance of aerosol transmission; however, the lack of objective and systematic evaluation of the methodology used in the selected studies precludes such usefulness. My major concern is that the review set out with the assumption that "the quality of transmission studies is known to be low" (reference #9 was also irrelevantly cited as described further below), although one of the main purposes of this review would be to evaluate the quality of evidence of each study. The benchmarks used to evaluate whether "Analysis & reporting outcomes (are) appropriate" (Table 3) were not described, but which were critical to evaluate the quality of each study. The assumption of air sampling studies were of poor quality in general can be felt along the manuscript, especially in Table 6 with a very large paragraph of criticism on Santarpia 2020, but personally I think a lot of evaluation on this particular study was misguided due to insufficient understanding of the methodology used

(as described further below). In some instances, studies were being described as poorly done without explanation of why the authors think so. I think including authors who have working knowledge in the field of air sampling studies and/or epidemiologic transmission studies would help to improve this review greatly.

Response:

Thanks for your observations. We have made some revisions.

We have revised the statement regarding reference #9.

"We simplified the tool because the included studies were not designed as primary diagnostic accuracy studies, and there is a lack of high-quality data in published transmission studies"

We have expanded the process used to assess the reporting quality in the methods section.

Our team includes viral transmission experts, epidemiologists, clinicians and systematic review experts. This review set out to assess whether published studies demonstrated evidence of SARS-CoV-2 transmissibility.

The included studies where the culture of viable virus was attempted were analysed in-depth given their importance and the potential for bias. We have considerable expertise in transmission studies. Across nine reviews we have assessed over 500 studies to date. CH and TJ are contact editors in the Cochrane Acute Respiratory Group and as a group, we have over 3 decades of experience in systematic reviews and infections. We, therefore, dispute this reviewer's assertion. We have clarified the methods as per the previous reviewer's response.

3. There are also quite a number of missing information in the Tables, truncated sentences and typos, which requires a thorough re-read and check.

Response:

We have re-read the manuscript to check for any grammatical errors and typos.

4. 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

For this definition of airborne (aerosol) transmission, emerging discussion has suggested aerosol transmission occurs in both short- and long-range (see my review).

Response:

Our definition was based on current WHO guidance. However, we have added a statement to reflect this view:

"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 However, some authors have defined aerosol transmission as occurring over both short and long distances (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7982882/>)."

5. A collection of particles (liquid or solid) ranging in size from 0.001 μm to over 100 μm suspended in a gas defines an aerosol.

Response:

We have revised our definition of aerosol (see response to peer reviewer 1 above).

6. Method

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.

Response:

We have revised the paragraph.

"We primarily included studies that reported sampling for the detection of SARS-CoV-2. However, we also included observational and randomised studies that investigated airborne transmission of SARS-CoV-2." Non-predictive and experimental studies were also considered for inclusion."

7. Studies should include sampling for the detection of SARs-CoV-2.

- Do you mean "air sampling for the detection of SARS-CoV-2"?

Response:

Thank you. Revised.

"air sampling for the detection of SARS-CoV-2"

8. 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.

Response:

Revised to "copies per m^3 of sampled air".

The included studies reported the total number of air samples as well as proportion of positive samples (if any). We presented the results as reported by the authors.

We have added the following footnote to table 1:

"For positive air sample proportions, the denominator refers to the total number of air samples as reported by the study authors irrespective of the method used for sampling."

9. Results

Limitations of the sampling methods and the poor-quality reporting make it difficult to discriminate between airborne or droplet nuclei transmission.

- Is "airborne" a typo here?

Response:

Thanks. Revised.

"The variation in sample methods coupled with flaws in the reporting make it difficult to distinguish between aerosol and droplet nuclei transmission."

10. 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.

Response: Revised. Deleted "and none were comparative".

"All the studies were observational."

11. Overall the reporting was heterogeneous.

- Do you mean the methods or the results in the reported studies were heterogeneous?

Response:

Thanks. We have revised the sentence. "Overall, there was heterogeneity in the methods used for air sampling across the studies."

12. 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).

Response:

The outdoors here refers to hospital outdoor environments. We have revised the statements.

"Hospital. There were 50 studies conducted in healthcare settings: 45 studies included binary RT-PCR air samples: 42 hospitals, 1 hospital outdoor environment, 1 hospital indoor and outdoor environment and 1 student healthcare centre." (revise figure 1).

"Of the studies conducted in the community, 15 were conducted in indoor settings: choir practice (2), care home (1), inside a bus (3), quarantine households (1), meat processing plant (1), block of flats (2), restaurant (3), buses and subway trains (1), and home residence (1); three studies were conducted in outdoor settings: public places (1), industrial outdoor (1) and outdoor of a working/residential area (1)."

13. (142 positives out of 1,403 samples: average 10.1%).

Response:

We have described the denominator above (total number of air samples irrespective

of sampling method).

14. 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 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).

Response:

Revised. Added a new sub-title.

"Indoors."

15. Discussion

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.

Response: We have emphasized the need for a framework to assess studies of transmissibility and included a reference.

16. Past attempts to detect infectious particles have proved difficult: aerosols are dilute and culturing fine particles is problematic.

- o Why is culturing fine particles (as opposed to coarse particles?) problematic, apart from being diluted?

Response: Thank you for raising this point. We have several references below which address this point. The reference by Verreault is one of the most comprehensive on this subject.

Verreault D, Moineau S, Duchaine C. Methods for sampling of airborne viruses. *Microbiol Mol Biol Rev.* 2008;72(3):413-444. doi:10.1128/MMBR.00002-08

Aaron J. Prussin, II, Linsey C. Marr, Kyle J. Bibby, *Challenges of studying viral aerosol metagenomics and communities in comparison with bacterial and fungal aerosols, FEMS Microbiology Letters, Volume 357, Issue 1, August 2014, Pages 1–9,*

see also Lednicky

Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients

[https://www.ijidonline.com/article/S1201-9712\(20\)30739-6/fulltext#](https://www.ijidonline.com/article/S1201-9712(20)30739-6/fulltext#).

'The amount of airborne virus detected per liter of air was small, and future studies should address (a) whether this is typical for COVID-19, (b) if this represented virus production relative to the phase of infection in the patient, (c) if this was a consequence of active air flow related to air exchanges within the room, (d) or if the low number of virus was due to technical difficulties in removing small airborne particles from the air.'

See also Collection, particle sizing and detection of airborne viruses

Pan A, Lednicky JA, Wu C.-Y. *International Journal of Infectious Diseases.* 100 (2020)

476–482

We have referenced Verreault D and Pan M as new references.

17. *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.’*

Response: We quote what Roy et al. reported in the New England Journal of Medicine who also states there is a “need for a better understanding of aerosol-acquired disease.’

18 *For coronaviruses, previous evidence supporting the airborne route of transmission is weak¹³.*
○ Please clarify that this review was published before the COVID-19 pandemic.

Response: Clarified: “For coronaviruses, previous review evidence supporting the airborne route of transmission is weak¹³; however, it should be noted that this review was published before the COVID-19 pandemic.”

19. *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.*

Response: We have deleted this statement within the manuscript.

20. *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.*

Response: Revised: “... ensure that there is evidence of exposure to a transmissible agent”

21. *No airborne study to date definitively demonstrates SARS-CoV-2 is of an infectious nature, which offers the most robust evidence of transmissibility²².*

Response: We have revised the statement. “None of the included studies definitively demonstrated that SARS-CoV-2 can be recovered in the air.”

22.
Table 1

Response: Thank you for pointing out the typographical errors and missing notes. We have made revisions to Table 1

**≥ 80 μm in diameter
The choir rehearsal room was not ventilated.
14 out of 56 samples**

Analysed outbreak using computer models and experiments based on airflow dynamics

Air-conditioned, 5-floor building without windows

It satisfied our inclusion criteria - observational studies of RCT that investigated airborne transmission

Revised; used quotation marks where necessary

Results added

Added

Added

Added

24. Figure 1

Response: For instance, several modelling studies did not 'study' a transmission outcome and were therefore excluded. This is different to whether studies 'demonstrate' an effect or not as both would be included irrespective of the result. Otherwise, we would introduce publication bias.

25. Figure 2

Response:

We have revised what we mean by follow-up. See methods section.

26. Table 4

Response:

Thanks. Revised. We have presented the units as reported by the study authors ($D < 0.056 \mu\text{m}$) up to coarse particles ($D > 18 \mu\text{m}$); as reported by the study authors No truncation. Sentence extends into next page

Corrected

Corrected

27. Table 5

Response:

No truncation; statement extends into the next page.

28. Figure 4

Response:

We have addressed this comment earlier.

29. Table 6

Response:

"A Ct of <20 would be considered positive..."

Corrected: Hu J 2020

30 Lednicky 2020a:

Response:

Revised: "The authors reported that plaque assays could not be performed due non-availability of the components which make up the culture media in the USA."

31. Santarpia JL 2020a:

Response: As far as we are aware, Santarpia 2020(a) is still only available as a preprint. The Scientific Reports citation is the journal publication for Santarpia 2020(b)

See the following reference. The reported changes in RNA were very small and may not be truly reflective of active replicating virus.

Jefferson T, Spencer EA, Brassey J, Heneghan C. Viral cultures for COVID-19 infectious potential assessment - a systematic review [published online ahead of print, 2020 Dec 3]. Clin Infect Dis. 2020;ciaa1764. doi:10.1093/cid/ciaa1764

'Complete live viruses are necessary for transmission, not the fragments identified by PCR. Prospective routine testing of reference and culture specimens and their relationship to symptoms, signs and patient co-factors should be used to define the reliability of PCR for assessing infectious potential. Those with a high cycle threshold are unlikely to have infectious potential.'

Thanks. We have deleted the statement changed text to:

"Neither plaque assay nor serial passage was attempted in the study."

Competing Interests: No competing interests were disclosed.

Reviewer Report 16 April 2021

<https://doi.org/10.5256/f1000research.55319.r82591>

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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, 2009¹) – are typically stated as being <100 μm 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}TCID₅₀ per liter of air. This reduction was similar to that observed with SARS-CoV-1, from 10^{4.3} to 10^{3.5}TCID₅₀ 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 Collision 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 viruses 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.

References

1. Tellier R: Aerosol transmission of influenza A virus: a review of new studies. *J R Soc Interface*. 2009; **6 Suppl 6**: S783-90 [PubMed Abstract](#) | [Publisher Full Text](#)
2. van Doremalen N, Bushmaker T, Morris D, Holbrook M, et al.: Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *New England Journal of Medicine*. 2020; **382** (16): 1564-1567 [Publisher Full Text](#)
3. Kutter J, de Meulder D, Bestebroer T, Lexmond P, et al.: SARS-CoV and SARS-CoV-2 are transmitted through the air between ferrets over more than one meter distance. *Nature Communications*. 2021; **12** (1). [Publisher Full Text](#)

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

If this is a Living Systematic Review, is the 'living' method appropriate and is the search schedule clearly defined and justified? ('Living Systematic Review' or a variation of this term should be included in the title.)

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.

Author Response 25 Aug 2021

Carl Carl

Peer reviewers' comments

Authors' responses

Peer Reviewer #1

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.

Response: 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, 2009¹) – are typically stated as being <100 μm 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 <5µm 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.

Response: We have revised the definition.

"There are varied definitions of aerosols in the published literature. An aerosol is defined as a collection of particles (liquid or solid) with varying aerodynamic diameters, suspended in the air (gas) for a prolonged period of time. The size of the particles and the distance they may travel is highly variable and depends on many factors,(

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2843947/>;

https://apps.who.int/iris/bitstream/handle/10665/112656/9789241507134_eng.pdf;jsessionid=41AA684FB64

)"Consider to add this reference Xie X, Li Y, Chwang AT, Ho PL, Seto WH. How far droplets can move in indoor environments--revisiting the Wells evaporation-falling curve. *Indoor Air.* 2007 Jun;17(3):211-25. doi: 10.1111/j.1600-0668.2007.00469.x. PMID: 17542834

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.

Response: We have revised the wording.

"Transmission via droplets and aerosols in specific settings or situations may potentiate the spread of some viruses in humans, resulting in disease outbreaks that are difficult to manage. The results of several studies investigating human-to-human virus transmission have been largely inconclusive, and the evidence to inform such transmission in experimental studies is often not available."

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.

Response: There is no Appendix 7 in the submission. However, see the response to comment 6 below where we expand on the methods used to assess reporting quality.

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.

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'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 $10^{2.7}$ TCID₅₀ per liter of air. This reduction was similar to that observed with SARS-CoV-1, from $10^{4.3}$ to $10^{3.5}$ TCID₅₀ 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 Collision 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 viruses 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.

Response:

We understand the reviewer's point involving the use of other methods to determine respiratory virus transmission. However, in our pre-specified "a priori" protocol, we planned to include sampling in the population or the environment. We do not discount the suggestion that SARS-CoV-2 can be sampled via other methods as the reviewer suggests. We have included this as a limitation of the study.

"We excluded study designs/settings that attempted to detect SARS-CoV-2 via other methods apart from air sampling, e.g., virus stability, outbreak reports, aircraft outbreaks, non-pharmaceutical intervention, experimental infection, air tracer studies and computational modelling/simulation"

Laboratory studies such as the one quoted provide insights into the stability of the virus in airborne suspensions but provide no insights into whether there exist ordinary biological mechanisms capable of generating such high-titer aerosols in the first place. The fact that one can put humans into orbit, doesn't mean it is an easily achieved or common task. It simply says humans can survive in orbit. The inclusion of laboratory studies was not a part of our protocol but could be included as a part of a separate review but is outside the scope of our study.

The suggestion to include animal models or laboratory-based studies, in general, would not be appropriate. An animal review would be a separate review with a specific methodology. The Collaborative Approach to Meta-Analysis and Review of Animal Experimental Studies (CAMARADES) research group aims to address the gap in systematic review and meta-analysis in this area. See: [CAMARADES | The University of Edinburgh](#).

We would like to point the classic 1964 Nature paper [Survival of Measles Virus in Air | Nature](#) (DE JONG, J., WINKLER, K. Survival of Measles Virus in Air. *Nature* 201, 1054–1055 (1964). <https://doi.org/10.1038/2011054a0>).

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.

Response:

We do consider a peer review seriously and do not seek to second-guess any conclusion but prefer to examine the evidence base in a rigorous manner. We have published over a hundred (100) systematic reviews and synthesize the evidence objectively in both this and a previous pandemic (see as an example: Neuraminidase inhibitors for preventing and treating influenza in adults and children Version

published: 10 April 2014 Version history
<https://doi.org/10.1002/14651858.CD008965.pub4>).

We analysed as in our previous work the published evidence to the date specified. We set out to determine whether SARS-CoV-2 could be detected in air samples. We have stated in our conclusion that the lack of positive samples does not rule-out airborne transmission and have tried to be as objective and open as possible but maintaining a rigorous evidence based approach.

The citation “**WHO Ebola 2014 IPC guideline**” may have been quoted out of context. Coronaviruses exhibit a variety of infection modes (respiratory, enteric, systemic), but if one looks beyond humans the disease is most commonly enteric in nature [see Saif (2004) Rev. sci. tech. Off. int. Epiz., 23 (2), 643-660]. The human respiratory strain OC-43 may have originated as a bovine enteric coronavirus. This is the reason why there has been so much interest in trying to detect and retrieve the SARS-CoV-2 from fecal specimens.

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.

Response: Thank you. We have expanded the section on the methods used to assess the quality of reporting.

“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 low⁹. We gave particular importance to the description of methods for air sampling and the reporting of sufficient detail to enable replication of the study. We examined the following domains: (i) Source population – did the study authors adequately describe the source population? E.g. setting, severity of SARS-CoV-2, baseline demographics including concurrent respiratory infections or other comorbidities, distance between study subjects; (ii) Methods – did the study authors sufficiently describe the methods used to enable replication of the study? E.g. methods used for diagnosing SARS-CoV-2 in patients, procedure used for air sampling, time-point for sampling, number of samples per site, cycle thresholds, culture methods, airflow/ventilation settings, humidity; (iii) Sample sources – did the authors clearly describe the sources for the air samples? What was the volume of air in each sample? Was the period of sampling similar across various sites? (iv) Outcome reporting – was the reporting of the results consistent with the study outcomes? Was the analysis of the results appropriate – e.g., interval and time-point for testing study participants for potential transmission; (v) Follow-up – was the pattern and number of air samples sufficient to demonstrate airborne transmission - e.g. repeat sampling, serial sampling?” The risk of bias for each domain was rated “low”, “moderate” or “high” depending on the adequacy of reporting. One reviewer (CJH) assessed the risk of bias while a second author (EAS) independently verified the risk of bias. Any disagreements were resolved through

discussion. Where a consensus could not be reached, a third reviewer (IJO) arbitrated.”

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.

Response: We already have a published protocol that has been used to conduct our series of systematic reviews of studies investigating transmission dynamics of COVID-19. However, our research is ongoing, the quality of the evidence and methods have changed over time and we make necessary adjustments to improve the robustness of the evidence as more studies (and evidence) become available (and examined). We are in contact with several original authors to clarify and update the methods.

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.

Response:

Thanks. We have made several revisions to improve the quality of the manuscript.

Competing Interests: No competing interests were disclosed.

Comments on this article

Version 2

Reader Comment 18 Sep 2021

Étienne Booth, Université du Québec à Chicoutimi, Saguenay, Canada

Heneghan et al's submission of *SARS-CoV-2 and the role of airborne transmission: a systematic review* in an Open Research publishing platform is commendable. It is in my opinion an encouraging sign that the authors are seeking transparent evaluation from competent peers from the scientific community.

I want to specify I am not one of those peers.

I do not hold the academic credentials required for such a title. My only claim to having any ability towards contributing something of value here is the fact that I have spent much of the past year working on an ongoing book project named *In Defense of Training*, which is on the subject of what

the SARS-CoV-2 pandemic has revealed about the place given to physical activity in society. Part of that project has required to immerse myself in the scientific literature regarding the risks of transmission during physical training.

In short, I am not commenting here as an academic or as an aerosol expert, but as a writer who has been interested in the broad subject of understanding and communicating the risks of SARS-CoV-2 transmission.

As such, I am limiting my comments to my level of competence and, to have a margin of safety, I am aiming to not go beyond college level science principles. By doing so, I by no means imply that Heneghan et al are not already expertly familiar with these. By basing my comments on fundamental principles, my goal is to distance myself from what I am not competent enough to have an opinion on, and try to contribute by pointing out what may simply be too obvious to be recognized.

Scientific thinking

At its core, scientific thinking is taking pertinent objective observations and using reason to draw out logical conclusions. Arguably one of the greatest traps of this process is cognitive dissonance and its bias manifestations, because they masquerade to its originator and unaware bystanders as logically coherent and scientifically valid.

As humans, we are all prone to logical inconsistencies because we harbor contradicting and often unconscious motivations. Given this reality, I am not negatively accusatory when I comment here that there seems to be cognitive dissonance and biases at work in Heneghan *et al's* publication. It simply means that scientists are human.

Coherent sequence of objectives

At the highest level, the greater objective of the WHO funded series of rapid reviews, of which Heneghan *et al's* publication is a part of, is stated as: "to undertake a series of living systematic searches and appraisal of evidence on SARS-CoV-2 modes of transmission and its related updates are informing WHO guidance and scientific documents." (Center for Evidence-Based Medicine, 2021)

At the level of this publication, the objective is "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." To Heneghan *et al's* credit, the scope of the publication's objective is very well communicated. It is broad and inclusive. It is also coherent with the greater objective of the WHO funded series.

Even more importantly, Heneghan *et al's* objective is worthy and necessary. It is meaningful. In the midst of this global pandemic, we need science to guide public health measures, which in turn guide individual actions. We need to understand if SARS-CoV-2 is transmitted through the air, and what factors increase or decrease the risk of such transmission.

At the title level, *SARS-CoV-2 and the role of airborne transmission: a systematic review*, is once again coherent with both the greater objective of the WHO funded living rapid review series and the specific objective from Heneghan *et al*'s publication.

Incoherent methodology with stated objectives

Together, all three levels (series, publication, title) form a consistent and logical sequence from the general to the specific. Broad in its scope. Inclusive in its search. Meaningful in its implications. But from that point, there are several logical inconsistencies within and between the methodology, discussion and conclusion.

Foremost, given the broad scope and inclusive search for evidence that is stated at all levels, it is hard to understand why Heneghan *et al* "excluded study designs/settings that attempted to detect SARS-CoV-2 via other methods apart from air sampling, e.g., virus stability, outbreak reports, aircraft outbreaks, non-pharmaceutical intervention, experimental infection, air tracer studies and computational modelling/simulation." As these studies have value towards attaining the publication's objectives, this is as logical as having the goal to "identify, appraise, and summarize all letters of the alphabet", while simultaneously excluding "letters B through Z".

Because of its overarching importance, I am reformulating here what commenter Jose-Luis Jimenez and reviewer Maosheng Yao have already put forward. Respectfully, there seems to be a logical disconnection between the scope of this publication's broad and inclusive objectives (at the series, publication and title level) and its narrow and exclusionary methodology.

To re-establish coherence, the publication could either:

1. Aim to review the evidence relating to the role of airborne transmission (and fulfil its practical mission of informing WHO guidance and scientific documents) and take in account all pertinent evidence.

OR

1. Aim to limit the review to evidence of RNA detection and viral culture of air samples, and change its stated objective, title and content to reflect this limited scope (but will be at odds with the overarching rapid review series mission).

Quite simply, the publication cannot logically be both. One option excludes the other. In its current form, the publication's duality of broad objectives coupled with its narrow methodology almost inevitably leads to a misinterpretation and overreach of Heneghan *et al*'s conclusions.

Rationalization of exclusions

It is especially incoherent to exclude, for example, laboratory and animal studies, then including in the discussion the Wells, Riley and Mills experiments (which are combined laboratory and animal studies) as a reference for the level of proof required to demonstrate airborne transmission:

“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” (Roy *et al.* 2004)

Furthermore, when reviewer David R. Tomlinson underlined the incoherence in version 1 of this publication, the authors’ response was: “The suggestion to include animal models or laboratory-based studies, in general, would not be appropriate. An animal review would be a separate review with a specific methodology.” As this does not address the core incoherence of excluding animal and laboratory studies, then including them as required proof in the discussion, this seems to be rationalization of cognitive dissonance.

Weighing evidence asymmetrically

A similar inconsistency appears at the end of Heneghan *et al.*'s discussion, where a paragraph is dedicated to studies for which the authors interpret the results as not supporting airborne transmission. Of the four studies cited, three are retrospective investigations of SARS-CoV-2 exposure that do not include air sampling for the detection of the virus (Bays D 2020, Mponponsuo K 2020, Wong SCY *et al.*, 2020). Inclusion of these studies as evidence against airborne transmission even if they should be excluded by the publication’s own methodology standards is incoherent. But excluding all other equivalent studies that could, by the same logic, be exposed as evidence in favor of airborne transmission, is the application of a double standard.

Hence, there is an asymmetry in how Heneghan *et al.*'s publication weighs and discusses evidence. In fact, the discussion only mentions studies that “do not support the airborne transmission hypothesis.” There is no mention that any study supports airborne transmission. Yet many of the studies reviewed by Heneghan *et al.* retrospectively investigated outbreaks in buses (Luo K 2020, Shen Y 2020), choirs (Charlotte N 2020, Hamner L 2020 and Miller SL 2020), a nursing home (De Man P 2020), a meat processing plant (Günther T 2020), an apartment building (Lin G 2020), and restaurants (Li Y & Qian H 2020, Lu J 2020), which conclude in favor of airborne transmission.

The selective inclusion in the discussion of studies concluding against airborne transmission, while excluding any mention of similar studies that conclude the opposite, is not only illogical, it is the text book manifestation of confirmation bias.

Strengths seen as limitations

Heneghan *et al.* are clearly correct in stating that “SARS-COV-2 RNA can be detected intermittently by RT-PCR in the air in a variety of settings”. That is an empirical fact. The stated “lack of recoverable viral samples” is beyond my own competence to comment on. But even without taking into account the technical issues commented by Raymond Tellier and Jose-Luis Jimenez regarding SARS-CoV-2 RNA detection and viral culture, there are several purely logical flaws in Heneghan *et al.*'s analysis of data.

First of all, absence of proof is not proof of absence. In this case, this is especially true for environments designed to dilute and evacuate airborne containments. Of the 42 indoor hospital

studies that included air sampling RT-PCR data, my own review showed that:

- 19 studies took samples in Airborne Infection Isolation Rooms (AIIRs) or in environments with equivalent or higher ventilation rates (12 air changes per hour (ACH) or more), although 3 of them were using methods to prevent dilution and evacuation of contaminants during sampling.
- 11 studies mentioned no ventilation rates, but had airborne infection mitigating measures such as being a negative pressure environment (usually, but not necessarily meaning at least 12 ACH), low CO2 measures (ranging from 341 to 503 ppm), being a “level 2 or 3 protection level”, having UV air disinfection or open windows during sampling.
- 7 studies mentioned no ventilation rates or airborne infection mitigating measure.
- 4 had relatively low stated ventilation rates (ranging from 1.5 to 7 ACH) and no mention of airborne infection mitigating measures.
- 1 Did not take air samples in rooms, but only in HVAC systems.

These numbers are not to be taken as absolute, as there is considerable overlap and grey areas in the methodology of each study (which is exactly Heneghan *et al*'s point). But what should be taken into account is that most studies were done in hospital environments with ventilation and or airborne transmission mitigating measures that do not represent the typical ventilation conditions of indoor environments in society.

Again, these are only estimations (true ventilation is usually based on occupant density and type of activity, as per ASHRAE 62.1, for example), but many homes will have a ventilation rate of around 1 ACH, offices and retail shops around 2-3 ACH, and restaurants around 6-8 ACH. And in most of these environments, the occupant density will be much higher than in an AIIR (where there is usually only one occupant). In consequence, any allusion that intermittent detection in the hospital studies goes against airborne transmission is tenuous.

Logically, it is to be expected that air samples taken in indoor environments engineered to dilute, evacuate or destroy airborne contaminants will have less chance of being positive than in indoor environments that are not. At a minimum, even intermittent positive detection in an AIIR or similar setting should be concerning, if not taken as a sign of increased risk of airborne transmission in less ventilated environments. Concluding otherwise is the logical equivalent of believing that there are no leaks because water is intermittently found at the bottom of boats with actively functioning bilge pumps.

A similar logical flaw seems to be made in Heneghan *et al*'s conclusion: “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”. Although this could be defended as being the statement of a fact, the phrasing implies that this should be considered evidence against airborne transmission. Again, absence of proof is not proof of absence. Finding positive surface samples (sometimes in unreachable ventilation ducts and filters) should logically lead to the question: “How did it get

there?”.

Yes, variable environmental conditions are stated by Heneghan *et al* as a limitation. But if the objective is not simply to suggest a standardised method of sampling and reporting, but to truly review evidence regarding the “role of airborne transmission of SARS-CoV-2 and the factors influencing transmissibility”, intermittent detection in settings designed to be unfavorable to airborne transmission should actually be considered as strength of evidence.

In my opinion, logically reviewing even the limited data considered by Heneghan *et al*'s methodology should not lead to a “eureka” against airborne transmission, but at a number of “that’s funny...” in favor of it.

Science is provisional

Up to this point, I have essentially used basic logical reasoning to analyse and comment Heneghan *et al*'s publication, mainly regarding its content. Now, I wish to shift to another basic scientific principle to analyse and comment on what the publication does not contain.

I need to underline that I understand that by setting viral culture of air samples as the “gold standard” of proof and by concluding that there is a need for standardised methods and improved reporting, Heneghan *et al*'s intention is to recognize nothing less than the direct and undeniable observation of infectious SARS-CoV-2 virus contained in expelled respiratory airborne particles by an index patient. There is nothing intrinsically wrong with this. High standards are commendable.

But this intention misses a fundamental principle and, by doing so, distances the publication from its functional objective.

Science is forever provisional on available data.

We formulate hypotheses and construct models to explain reality, and these must be changed when new data disconfirms them. Although models are inherently imperfect (the map is not the territory), they are still useful. As such, action based on science is using the best available model, the one that best fits our empirical observations of reality, even if direct proof has not been observed.

If the map works, it is better to use it than flying blind.

So, what Heneghan *et al*'s publication is missing is the mention that airborne transmission is the best model humanity has to explain and combat the SARS-CoV-2 pandemic, even if viral cultures from airborne samples were to be discarded.

It also does not mention the comparative weakness of any alternative model of transmission, all of which do not hold up to any practical comparison to the empirical observations accumulated after nearly two years of this global pandemic.

The streams of evidence supporting this claim have been very well summarized in the peer-

reviewed Lancet commentary from Greenhalgh *et al.* Many of these were brought to the attention of Heneghan *et al.* by the comments of Jose-Luis Jimenez on version 1 of their publication, such as:

- Animal studies showing airborne transmission (e.g. Kutter *et al.* 2021).
- Long distance transmission (e.g. Katelaris *et al.* 2021).
- Transmission is twenty times more frequent indoors than outdoors (Bulfone *et al.* 2021). This cannot be explained by large droplet transmission, but is readily explained by airborne transmission, due to much higher dispersion outdoors than indoors.
- Superspreading events appear to occur dominantly in poorly-ventilated indoor spaces. This has led e.g. WHO to recommend ventilation as a way to reduce transmission, including detailed guidelines (World Health Organization 2021). This again is easily explained by airborne transmission, but not by large droplets or fomites which are not substantially impacted by ventilation.
- Presymptomatic and oligosymptomatic transmission is known to occur (Johansson *et al.* 2021), and may be an important reason why we are in such a difficult-to-control pandemic. Measurements show that people without cough produce few droplets but abundant aerosols, (Chen *et al.* 2020), favoring the airborne route of transmission for people without a cough.
- Nosocomial infections have been reported in several studies, including some showing genomic match, despite wearing of surgical masks and eye protection (Klompas, Baker, Griesbach, *et al.* 2021; Klompas, Baker, Rhee, *et al.* 2021; Goldberg *et al.* 2021).
- SARS-CoV-2 virus material has been measured (by PCR) after sampling ducts and filters in a hospital building (Nissen *et al.* 2020). It could only have reached these locations as an aerosol.

Airborne transmission elegantly fits all of the above streams of evidence. All other transmission theories do not.

Science is unconstrained to a specific discipline

In the same line of thought, I am adding a final principle; science is not constrained to a specific discipline. It gains by being open. A theory that holds up against the basic models of physics, engineering, biology and medicine has a better chance of surviving the test of reality than if it is isolated in the theoretical vacuum of a single discipline.

The alternative SARS-CoV-2 transmission theory of combined ballistic droplets and fomites as main drivers of the pandemic can only live in the theoretical vacuum of historically accepted medical norms. It does not hold up to the previously stated streams of evidence. In fact, it is incoherent with even some of the most basic models of science:

- The ballistic droplet theory excluding particles >5-10 μm from airborne transmission, as stated in Appendix 1, goes against Newtonian physics. In reality, particles up to around 100 μm can remain suspended long enough to be transported in the air and inhaled, the 5-10 μm limit being a historical mix up with the particles able to reach the deep lung (Randall *et al.* 2021).
- As David R. Tomlinson pointed out in his version 1 review, to the best of humanity's understanding of biology, viruses are not sentient. They cannot choose to be contained only in respiratory particles that do not remain suspended in the air.
- As Jose-Luis Jimenez commented on version 1 of this publication, ballistic droplets and fomites cannot explain superspreading events that occur exclusively in poorly-ventilated indoor environments, but the basic engineering models of fluid dynamics can (e.g. Lewis 2021 and references therein, Hamner L 2020).

So, for the alternative theory of ballistic droplets and fomite transmission to be true, it requires us not to compare it to numerous streams of evidence and basic models coming from multiple scientific disciplines. Fundamentally, it requires us not to observe or think. That is a bad sign.

What is missing in Heneghan *et al's* publication is the consideration that airborne transmission becomes more robust as you compare it to the basic models of different scientific disciplines.

Belief Perseverance

The other telltale signs of a dysfunctional theory or model is the necessity of adding exceptions, ignoring contradicting observations, or explaining them in an increasingly improbable way in order to fit reality. Individually or combined, the direct contact, fomite and ballistic droplet theories require all of these.

Confirmation bias is the often-unconscious search and inclusion of evidence in favor of an initial hypothesis, while also unconsciously missing or misinterpreting evidence against it. But, once disconfirming evidence is clearly presented, refusing to take these into account becomes a conscious, intentional affair.

There comes a point where consciously refusing credible evidence becomes belief perseverance, a bias so great that no contradicting proof can change the believer's perspective.

Ignaz Semmelweis proved with a simple hand washing protocol that unclean hands were the source of many post partum infections, even before the bacteria responsible could be observed. John Snow did the same regarding the propagation mode of cholera through his famous pump handle removal of a fecal contaminated water source. William Wells proved airborne transmission of tuberculosis with an experiment using logic and reason, not by bacterial culture from air samples.

In all of these historic cases, the evidence was for many years deemed unconvincing, of low quality. But what truly prevented acceptance (and has led to unnecessary death) was not the lack or the

quality of evidence, but the perseverance of strongly held beliefs.

Contrary to these examples, where only a few individuals were toiling away to produce a single piece of proof against a dominant belief, the SARS-CoV-2 pandemic has brought the whole world's scientists together in producing enormous amounts and diversity of evidence.

Viral culture from airborne samples could be completely discarded as an evidence stream, it would not change the overwhelmingly coherent sum of all other empirical evidence in favor of airborne transmission and the comparative weakness of alternative theories.

If this fact is being ignored, even after being brought forward numerous times by commenters and reviewers, it would be an indicator that belief perseverance is at work as a bias in Heneghan *et al's* publication.

Conclusion

Given some of the fundamental principles that science is:

1. Using reason to draw out logical conclusions.
2. Provisional, yet we should still use the best available model.
3. Not constrained to a specific discipline.

I respectfully encourage Heneghan *et al's* to wonder what the great scientific minds that they themselves respect and admire would do with the relevant empirical data that has been accumulated after nearly two years of this worldwide pandemic. What would these people of reason do with what has been commented here (by other more competent minds than me) as credible arguments in favor of airborne transmission, especially considering the comparative weakness of any evidence for an alternative mode of transmission?

Although the process of eliminating biases in the pursuit of truth is central to the role of a scientist, it still takes great effort, strength and courage to recognize and untangle them. In fact, it is sometimes so difficult that, as Max Planck has said, only the passage of time leads to acceptance :

"A new scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die and a new generation grows up that is familiar with it."

I truly hope, for humanity's sake, that we will all prove him wrong.

Étienne Booth

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Competing Interests: No competing interests

Version 1

Reader Comment 18 May 2021

Raymond Tellier, McGill University, Montreal, Canada

Regarding the review in Heneghan *et al.* of Lednicky 2020a [1], which reported successful isolation in cell culture of SARS-CoV-2 from aerosol samples: Heneghan *et al.* take issues with the following:

- 1) No plaque assays was performed to estimate the concentration of viable viruses in the air due to shortage of some reagents, and a TCID₅₀ based assay was performed instead.
- 2) The cytopathic effect (CPE) was observed 6 to 11 days post inoculation.
- 3) No serial subcultures were done.
- 4) Infection of susceptible animals with cultured isolates was not performed.
- 5) There was no attempt to demonstrate a gradient effect by collecting aerosol samples at different distances from the source patient.

These criticisms appear very unconvincing.

1) Infectious titration by plaque assay typically requires a soft agar overlay; I would speculate that soft agar was the reagent unavailable as there is a shortage of several agarose products. At any

rate there is nothing wrong with an infectious titration done by the TCID method, which is a standard and well established method in virology and indeed essentially equivalent to the plaque assay for viruses that produce plaques (one TCID₅₀ is approximately 0.69 plaque forming unit [PFU]). We note that many other authors have used TCID-based titration for SARS-CoV-2 (e.g., [2]).

2) *“With the exception of a few slower-growing viruses such as CMV, or when viruses are present at very low titers, the time to detection by traditional tube culture method is generally between 1 and 7 days of inoculation”* [3].

As such, observing CPE 6 to 11 days following inoculation of a low viral load sample is therefore not unexpected. Other authors have used an observation period of 14 days for CPE when attempting to culture SARS-CoV-2 from samples with a very low viral load [4]. We would also note that although the complete CPE with rounding and detachment of cells took 6 to 11 days, early CPE foci with vacuolisation were already noted by 4 to 6 days [1].

3) The implied requirement for serial passage looks very much like an attempt to move the goalpost for acceptance of the existence of infectious SARS-CoV-2 viruses in aerosols. If the isolate would have been passage N times, would there have been a request to passage it (N+1) times?

Primary isolates from clinical samples are not laboratory adapted strains; they typically require specific mutations to adapt to the cell lines and when dealing with a low viral load sample the adaptation and the serial passage may or may not be successful; this does not negate the reality of infectious viruses being present in the sample. It has been reported that SARS-CoV-2 undergo adaptation in Vero E6 cells for at least 5-6 passages, with an increasing viral titer, acquisition of a large plaque phenotype and deletions in the furin cleavage site [5, 6].

4) Likewise, the implied requirement for successful animal infection with isolates recovered from air samples looks like another attempt to move the goalpost, and is especially surprising given that, as noted by the Reviewer #1, Heneghan *et al.* did not include in their review animal transmission studies.

5) Why would the demonstration of a spatial gradient be considered essential? To be sure a greater concentration of aerosols near the source is to be expected but the steepness of the gradient would be modulated by the specific ventilation characteristics of the room. There would be also stochastic effects since an essential property of aerosol is that they will disperse randomly, not uniformly [7]. But in the end the important result is the demonstration of infectious viruses in aerosol particles.

We find all of the above disquieting in a review that aspires to be seen as authoritative, as it strongly suggests a lack of familiarity with the methods of Clinical Virology and the problems involved in culturing viruses present in very low viral load in clinical samples.

One may ask, of what benefit can it be to have a review of a very large number of published studies if one cannot trust that the studies have been summarized accurately and knowledgeably evaluated?

Raymond Tellier McGill University
Julian W. Tang University of Leicester

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Competing Interests: No competing interests were disclosed.

Reader Comment 14 May 2021

Jose-Luis Jimenez, University of Colorado-Boulder, USA

Heneghan *et al*'s paper is not, as it claims, a systematic review on the role of airborne transmission for SARS-CoV-2 (Heneghan *et al.* 2021). The mismatch between the paper's title, scope, methodology and conclusions could potentially misinform global policy and cost many lives.

The authors, who do not appear to have extensive (or perhaps any?) empirical experience in sampling viruses from the air, judge all the studies they reviewed to be of "low quality." Aerosol scientists would not reach the same conclusions. Indeed, a study of viable influenza virus released in aerosol particles during coughing and breathing (Lindsley *et al.* 2016) illustrates a number of quality criteria which a reviewer with expertise in aerosol science would expect of an empirical study in the field, namely the use of a well-characterized sampling system, detailed description of the sampling medium, careful accounting of collection efficiency as a function of particle size, sample replication, and purging to prevent cross-contamination. Heneghan *et al*'s technocratic application of a 'risk of bias tool', QUADAS2, that was developed in a completely different discipline

(clinical epidemiology) for assessing studies of an entirely different kind (diagnostic accuracy studies) has led these authors to misclassify many excellent studies. This is a troubling example of what has been termed “epistemological trespassing,” and could have been avoided had the review team included an expert from the relevant academic field. Indeed, we are surprised that the World Health Organisation did not require some minimum level of topic expertise when commissioning the review.

A comprehensive review on the question of airborne transmission would need to include a much wider range of types of evidence. Importantly, airborne transmission of other diseases was accepted mainly on the basis of types of evidence not included in the Heneghan review, such as:

- Tuberculosis: animal studies (Riley *et al.* 1962)
- Measles: efficacy of UV disinfection (Wells 1943) and superspreading events with long-distance transmission (e.g. Bloch *et al.* 1985)
- Chickenpox: superspreading events (Leclair *et al.* 1980)

The type of evidence reviewed by Heneghan *et al.*, namely demonstrating the infectivity of pathogens captured from the air, has never been achieved, to our knowledge, for tuberculosis, measles, or chickenpox (Morawska and Milton 2020; Fennelly 2020). Therefore if a review of only this narrow type of evidence allows one to reach the conclusion that “The lack of recoverable viral culture samples of SARS-CoV-2 prevents firm conclusions over airborne transmission,” as done in this paper, we must reach the same conclusion for tuberculosis, measles, and chickenpox. This would clearly be nonsensical. Heneghan *et al.* have thus applied a much higher standard of proof to SARS-CoV-2 than to other pathogens that are widely agreed to be airborne. There is no discussion in the Heneghan paper about why such a higher standard of evidence is needed only for SARS-CoV-2, and of why the other types of evidence that led to the acceptance of other diseases as airborne have not even been considered.

Indeed, the types of evidence that led to the acceptance of tuberculosis, smallpox, and measles as airborne are available for SARS-CoV-2:

- Animal studies showing airborne transmission (e.g. Kutter *et al.* 2021)
- Superspreading events that occur exclusively in poorly-ventilated indoor environments, and can be generally explained by airborne transmission but not large droplet or fomite transmission, (e.g. Lewis 2021 and references therein; Miller *et al.* 2021)
- Long distance transmission (e.g. Katelaris *et al.* 2021)

In addition, as recently summarized in a peer-reviewed commentary in *The Lancet* (Greenhalgh *et al.* 2021), there are multiple additional streams of evidence that are also not considered at all by Heneghan *et al.*, and that also support airborne transmission of SARS-CoV-2. These include:

- Transmission is twenty times more frequent indoors than outdoors (Bulfone *et al.* 2021). This cannot be explained by large droplet transmission, but is readily explained by airborne transmission, due to much higher dispersion outdoors than indoors.
- Superspreading events appear to occur dominantly in poorly-ventilated indoor spaces. This

has led e.g. WHO to recommend ventilation as a way to reduce transmission, including detailed guidelines (World Health Organization 2021). This again is easily explained by airborne transmission, but not by large droplets or fomites which are not substantially impacted by ventilation.

- Presymptomatic and oligosymptomatic transmission is known to occur (Johansson *et al.* 2021), and may be an important reason why we are in such a difficult-to-control pandemic. Measurements show that people without cough produce few droplets but abundant aerosols, (Chen *et al.* 2020), favoring the airborne route of transmission for people without a cough.
- Nosocomial infections have been reported in several studies, including some showing genomic match, despite wearing of surgical masks and eye protection (Klompas, Baker, Griesbach, *et al.* 2021; Klompas, Baker, Rhee, *et al.* 2021; Goldberg *et al.* 2021).
- SARS-CoV-2 virus material has been measured (by PCR) after sampling ducts and filters in a hospital building (Nissen *et al.* 2020). It could only have reached these locations as an aerosol.

Therefore, it is clear that there are many more lines of evidence to consider in order to ascertain the importance of airborne transmission of SARS-CoV-2. It is well-known that viruses are fragile and they are easily damaged when sampling them from the air (Pan *et al.* 2017), leading to difficulties in detecting viable virus. Amounts of pathogens in air that scientists have so far failed to cultivate can still drive disease transmission, as exemplified by measles and tuberculosis. Studying only the narrow topic of cultivation of viruses from air samples is therefore likely to give a highly misleading picture of the relevant evidence base.

The authors also appear to expect that live virus should be detectable in all locations where COVID-19 patients are present. However, it is clear that viral load is extremely variable both in time (He *et al.* 2020) and between people (Yang *et al.* 2021). Aerosol generation is also highly variable among different people (Asadi *et al.* 2019; Edwards *et al.* 2021). Measurements of viral load by PCR in exhaled breath showed 73% of COVID-19 patients did not produce detectable virus in their breath, but 27% exhaled, on average, millions of virus copies an hour (Ma *et al.* 2020). The very high variability in the amount of exhaled virus may apply to other diseases, and perhaps explain cases of lack of infection with shared air, that were used to deny the airborne character of measles for 7 decades (Bloch *et al.* 1985). This variability in virus emission may be a key reason why some people transmit SARS-CoV-2 to lots of others, while others do not transmit to anyone (Sun *et al.* 2021; Endo *et al.* 2020). In addition, ventilation of indoor spaces varies substantially across different environments and will lead to very variable amounts of dilution of any exhaled virus across the studies. Given all of these considerations, a high variability in the amount of virus captured from the air is not just not surprising but expected, and it is not an argument against airborne transmission of SARS-CoV-2.

In summary, no conclusions can be made about the importance of airborne transmission from only this review, while excluding all the other lines of evidence. We caution against using the findings of this review to inform policy.

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