



# Molecular detection of SARS-CoV-2 from indoor air samples in environmental monitoring needs adequate temporal coverage and infectivity assessment

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## ABSTRACT

The relevance of airborne exposure to SARS-CoV-2 in indoor environments is a matter of research and debate, with special importance for healthcare low-risk settings. Experimental approaches to the bioaerosol sampling are neither standardized nor optimized yet, leading in some cases to limited representativity of the temporal and spatial variability of viral presence in aerosols. Airborne viral viability moreover needs to be assessed. A study has been conducted collecting five 24-h PM10 samples in a COVID-19 geriatric ward in late June 2020, and detecting E and RdRp genes by RT-qPCR with a Ct between 36 and 39. The viral RNA detection at Ct = 36 was related to the maximal numerosity of infected patients hosted in the ward. Lacking a direct infectivity assessment for the collected samples an experimental model has been defined, by seeding twelve nasopharyngeal swab extracts from COVID-19 positive patients on Vero E6 cells; only the four extracts with a viral load above E+10 viral copies (approximately Ct<24) have been able to establish a persistent infection *in vitro*. Therefore, the cytopathic effect, a key feature of residual infectivity, could be considered unlikely for the environmental PM10 samples showing amplification of viral RNA at Ct = 36 or higher. A standardization of airborne SARS-CoV-2 long-term monitoring and of environmental infectivity assessment is urgently needed.

## 1. Introduction

Healthcare facilities hosting patients affected by coronavirus disease 2019 (COVID-19) are critical hotspots for potential infection and diffusion of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-

CoV-2), due to the possible viral spread through respiratory or aerosol droplets (Tellier et al., 2019; Razzini et al., 2020; Lednický et al., 2020; Chia et al., 2020; Milton, 2020); the relevance of infection by fomites has been recently reduced (Goldman, 2020). Respiratory droplets are usually defined as drops characterized by a diameter >5 µm and with a

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range of dissemination of 1 m, thus being able to transfer virions to surfaces generating fomites. Another way of transmission that needs to be considered is the airborne way of infection (Morawska and Cao, 2020; Morawska and Milton, 2020) through aerosol droplets that are particles presenting a diameter  $<5 \mu\text{m}$  with a potential release of several meters for prolonged time (minutes until hours) (Sommerstein et al., 2020; Setti et al., 2020a).

Notably, the  $5 \mu\text{m}$  threshold has been questioned (Prather et al., 2020; Buonanno et al., 2020), and relevance of bioaerosol particles of smaller size - constituting the so-called inhalable fraction of particulate matter having long residence time in air - in the COVID 19 spread is supported (Lee, 2020) with implications for the healthcare workers protection in both low and high risk settings (Zhang et Duchaine, 2020). SARS-CoV-2 airborne transmission in close environments is still a subject of debate in the scientific community and further research efforts are needed to completely clarify this issue, despite general evidence of potential effects (Wei and Li, 2016).

As a matter of fact, several sampling approaches and devices are available for the collection and detection of airborne viruses (Rahmani et al., 2020; Pan et al., 2019). In a recent work, Comber et al. (2020) reviewed 16 articles reporting results about the collection of air samples and associated SARS-CoV-2 detection in healthcare facilities. Intensive care units (ICU) have been often considered, while less attention has been addressed to low risk settings or to general wards. Examining the methods used in the literature, it can be observed that there is a broad variety of combination of the following experimental setup parameters: sampling devices, supports for the sample collection, sampling time, air flow, sampler positioning, number of days of repeated sampling, time/s of the day selected for sampling, number of samples collected repeatedly at the same sampling point, SARS-CoV-2 detection methods. Nine studies (Chia et al., 2020; Guo et al., 2020; Jiang et al., 2020; Lei et al., 2020; Razzini et al., 2020; Santarpia et al. 2020a, 2020b; Zhou et al., 2020) present in the above mentioned review as also further studies published (Ding et al., 2021; Feng et al., 2021; Kenarkoohi et al., 2020) found from 3.6% to 100% samples positive for SARS-CoV-2 RNA with a total number of samples collected ranging from six to sixty-five (see Table SM1 in the Supplementary Material for details), having shown feasibility of SARS-CoV-2 RNA detection from environmental samples by means of several different experimental set ups.

In general, sampling procedures have been characterized till now by short time (tens of minutes to few hours) and low volume of aerosol collection (hundreds of liters to few cubic meters), but this kind of spot assessment appears a hardly representative monitoring for the environmental exposure scenarios in healthcare units classified as low risk settings; they can receive patients initially in not critical conditions, with evolving viral charge. The presence of airborne virus can be missed due to inadequate temporal coverage for capturing variability in virion emissions from patients and due to inadequate sample size.

When looking at outdoor studies, Setti et al. (2020b) found SARS-CoV-2 RNA in  $\text{PM}_{10}$  daily filters (24h at  $2.3 \text{ m}^3/\text{h}$ ) sampled before mitigation effects of lockdown containment measurements at an Italian industrial site, in the peculiar winter environmental conditions of the Po Valley (low mixing height and high particle numbers). Hadei et al. (2021) detected SARS-CoV-2 RNA in public places and transportations on TSP on PTFE and glass fiber filters for shorter times. These suggest an opportunity for considering 24 h/full day samplings on filtering membranes also in indoor studies on airborne viral RNA.

Indeed, beside the studies on the viral RNA presence in aerosols, it is important to assess the potential infectivity of the collected samples. In this way, Comber et al. (2020) also presented four studies with infectivity assessment but in laboratory controlled conditions, where aerosols with SARS-CoV-2 were experimentally generated and the viral viability and integrity were investigated in different experimental settings. On the other hand, few studies tried to cultivate the virus from the aerosol, Zhou et al. (2020) and Binder et al. (2020) failed in recovering infectious air samples, meanwhile Lednicky et al. (2020) and Santarpia et al.

(2020a, 2020b) found evidence of SARS-CoV-2 amplification on Vero cells infected with air samples collected in real indoor environment. Even if airborne SARS-CoV-2 RNA detection on indoor particulate matter (PM) can be a tracer/indicator for potential exposure, viral RNA presence in the environment does not necessarily imply infectivity and thus effective risk of infection (Comber et al., 2020); so virus viability needs to be assessed. To date, since a universal consensus regarding the infectious potential of airborne SARS-CoV-2 is not achieved, precautions and preventive measures need to be undertaken (e.g. social distancing, facial masks), moreover, this holds in first instance for the safeguard of healthcare and medical personnel. After a review on studies related to health care worker protection, Zhang and Duchaine (2021) proposed a model of airborne transmission of SARS-CoV-2 involving inhalable particles (defined as the fraction of particles capable of penetrating into the head airways or below, upon inhalation (Milton, 2020): it excludes larger ballistic droplets but it includes particles larger than  $5 \mu\text{m}$ ) and giving little relevance to thoracic and respirable particulates. The coarse fraction of  $\text{PM}_{10}$ , can settle in the nose area rich in ACE-2 receptors and thus be considered as a relevant vehicle for SARS-CoV-2 infectivity, providing hints for aerodynamic size selection in sampling, and supporting statistical observational finding stronger correlation between COVID-19 outcomes in Italy during early stage of the pandemics with  $\text{PM}_{10}$  rather than with  $\text{PM}_{2.5}$  (Setti et al., 2020c).

Beside environmental monitoring of viral RNA presence, sound and informative risk assessment on SARS-CoV-2 transmission requires an infectivity assay that - at the present state of the knowledge - is performed by testing the potential infection on Vero E6 cells in BSL3 lab, directly, for available fresh environmental aerosol samples, adequately collected in liquids with dedicated instrumentation (Pan et al., 2019; Lednicky et al., 2020), up to now not commonly available among hygiene nor environmental sampling professionals.

In the present work, results are reported from a short-term air monitoring campaign conducted in the tail of the first COVID-19 wave in Italy (June 2020) at a geriatric COVID-19 ward of the Ospedale Maggiore in Trieste (Italy). The findings have been interpreted based on the comparison of cycle threshold (Ct) from air monitoring with Ct and viral copies quantification obtained by infecting Vero E6 cells with clinical oral/nasal swabs specimens positive for SARS-CoV-2 RNA at the diagnostic analysis, observing the presence/absence of cytopathic effects on Vero E6 cells exposed to the clinical samples. Therefore, we show an indirect method for sampling - schemes not having implemented samplers dedicated to preserve virus viability, that could be used also for future epidemics.

Cycle threshold from air samples (Ct-as) can be compared to cycle threshold from lowest virion concentration - obtained by dilution of extracts from oral/nasal swabs collected from infected patients (Ct-dpt) - that provokes cytopathic effects on Vero E6 cells in BSL3 lab; if Ct-as exceeds Ct-dpt air samples have the potential of being infective.

With the aim of supporting an assessment of potential infectivity for daily environmental samples, a model study has been designed and performed, correlating the Ct of SARS-CoV-2 RNA from air sample, indicative of the quantity of nucleic acid present, and the viral load of nasopharyngeal samples collected in clinical practice with the results from infectivity assay on Vero E6 cells.

## 2. Materials and methods

### 2.1. Sampling site description

In order to assess feasibility of airborne RNA detection in healthcare indoor settings with daily coverage, aerosol potentially containing SARS-CoV-2, was collected on glass fiber filters with size selection as  $\text{PM}_{10}$  operating daily for 24 h, in a monitoring activity from 19 to June 23, 2020, at the Geriatric ward of the Integrated University Health Authority Giuliano-Isontina (ASUGI) - Maggiore hospital of Trieste (Italy).

The Geriatric ward became a COVID-19 healthcare unit in late March 2020. During the pandemic emergency period it had dedicated and separated entrance and exit rooms that allow dressing of operators with personal protective equipment and access to the main corridor, and 13 rooms hosting COVID-19 patients.

A map of rooms of the Geriatric ward with the sampler position is reported in the supplemental materials (fig. SM1). Table 1 reports for each sampling day, the number of positive patients in the Geriatric unit, the number of hosts with first diagnosis recent more than 10 days, the number of hosts having less than 10 day from first positivization, the number of hosts re-positivized after negativization.

## 2.2. Air sampling

PM<sub>10</sub> has been 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 (de Gennaro et al., 2015). One PM sample (24 h for a total of 14.4 m<sup>3</sup> of air) was collected every day. The Silent sampler has been located at a middle position along the main corridor of the ward (see supplemental materials), with PM<sub>10</sub> sampling head at 150 cm from the floor. The sampling has been performed from 19 to June 23, 2020 just before the closure of this COVID-19 unit, in the tail of the first wave of the infection in Italy.

Sampling heads containing PM<sub>10</sub> on filters were brought in sealed bags to the BSL2 lab of the Functional Genomic Research Unit of the University of Trieste for SARS-CoV-2 molecular detection.

## 2.3. SARS-CoV-2 detection on air samples

RNA from the filters has been extracted using the ZymoBIOMICS RNA Miniprep Kit Zymoresearch, Irvine, CA, USA), modified as reported in (Setti et al., 2020b). From half filter, using 1 ml of lysis buffer, it was possible to recover about 400 µl of solution which was then processed according to the protocol with a final eluate of 50 µl.

In parallel, we processed 1 brand-new spiked filter with 10<sup>6</sup> synthetic RNA molecules and 2 filters with environmental PM<sub>10</sub> for air-quality monitoring spiked with 10<sup>6</sup> and 10<sup>8</sup> synthetic viral RNA molecules, to verify the efficiency of the extraction method. The synthetic viral RNA was kindly provided by Institute Pasteur, Paris.

For the assessment of viral RNA, given the particular origin of the sample, of qScript XLT 1-Step RT-qPCR ToughMix, presenting a DNA polymerase able to be efficiently processive even in the presence of inhibitors (Quantabio, Beverly, MA; USA) has been used. Five µl of RNA were amplified in a final volume of 12.5 µl, with the Ip2 primers and probe for region 1262190-127297 of the RdRp gene, (600 nM primer forward GTGARATGGTCATGTGTGGCGG, 800 nM primer reverse CARATGTTAAASACACTATTAGCATA, 100 nM probe FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ, nucleotide numbering based on Wuhan-Hu-1 MN908997, Institute Pasteur, Paris) on the CFX

**Table 1**

Positive patients at the Geriatric Healthcare Unit during the sampling days.

Sampling date	Number of positive patients	Host(s) having less than 10 days from first positivization	Re-positivized hosts
June 19, 2020	7	1	1
June 20, 2020	5	–	2
June 21, 2020	5	–	2
June 22, 2020	5	–	2
June 23, 2020	5	–	2

connect Real Time PCR detection system (BioRad, Hercules, CA, USA) in 4 replicates with the following protocol: 55 °C for 20', 94 °C for 3', then 50 cycles with a step at (94 °C for 15 '' and a step at 58 °C for 30 '').

## 2.4. Swab sampling

Twelve clinical swabs were collected from 12 COVID-19 patients enrolled at the emergency room (ER) of the Monfalcone hospital (ASUGI, Italy). All patients showed positivity for SARS-CoV-2 infection, after undergoing the molecular diagnostic test employed in the routine diagnostic practice of the Monfalcone hospital laboratory (Allplex SARS-CoV-2 assay, Seegene, Seoul, South Korea on the CFX connect Real Time PCR detection system, BioRad, Hercules, CA, USA). The swabs were preserved in universal transport medium (UTM) at 4 °C until processed, 2 days after the collection.

## 2.5. Infectivity assay on Vero cells

Vero E6 cells, a permissive epithelial normal cell line derived from kidney of *Cercopithecus aethiops* (ATCC CRL-1586), were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 8 mM glutamine, 100 U/ml penicillin 100 mg/L streptomycin (Euroclone, Pero, Milan, Italy). The day before the infection, the cells were seeded at a confluence of 150.000 cells for each well of a 12 multi well plate (Corning, New York, USA).

Briefly, the swab samples were filtered by using a 0.22 µm filter (Sarstedt, Nümbrecht, Germany) in order to remove cellular debris, mucus, bacteria and fungi (La Scola et al., 2020). Then, 500 µL of the specimens were seeded on a monolayer of Vero cells and 3.5 ml of infection medium were added (MEM +2% FBS, 8 mM glutamine, 100 U/ml penicillin, 100 mg/L streptomycin, and 2.5 µg/ml amphotericin B (Euroclone, Pero, Milan, Italy)).

The viral load was assessed in the supernatants at day 0, 2, 5 and 7. For each well 200 µL of the supernatants were collected, and the RNA extracted by using the Zymo RNA extraction kit, following manufacturer's instructions (Zymo Research, Irvine, CA, USA). The viral load has been quantified using the CDC primers and probe for N gene (nucleocapsid, 500 nM forward primer GGG AGC CTT GAA TAC ACC AAA A, 500 nM reverse primer TGT AGC ACG ATT GCA GCA TTG, 125 nM probe FAM-AYC ACA TTG GCA CCC GCA ATC CTG-BHQ1), with the Luna Universal Probe One-Step RT-qPCR Kit (New England Biolabs, Ipswich, MA, USA) on the 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) with the following amplification protocol: 50 °C for 10', 95 °C for 1', and then 40 cycles at 95 °C for 10'', 60° for 30''. The nCoV-CDC-Control Plasmid previously quantified (Eurofins, Luxembourg) was employed to generate the standard curve.

At the end of the 7 days, the cells were fixed in paraformaldehyde 4% in phosphate buffer saline (PBS) for 20', and then stained with the crystal violet 10% in PBS for 30' in order to visualize the possible cytopathic effect due to virus infection.

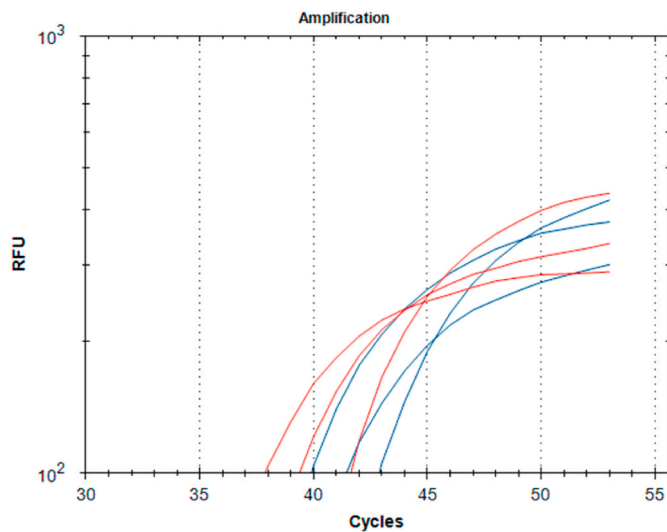
## 3. Results and discussion

### 3.1. Indoor air sampling

Six quartz fiber filters dated 19, 20, 21, 22 and June 23, 2020 of daily (24 h) samples of atmospheric particles were collected in the corridor of the Geriatric ward at the Integrated University Health Authority Giuliano-Isontina (ASUGI) - Maggiore hospital of Trieste (Italy).

### 3.2. SARS-CoV-2 RNA detection on indoor air samples

The very first amplification curves of RdRp gene from RT-qPCR obtained from the 5 collected samples (19–23 June 2020) are shown in Fig. 1. The analysis includes, as reference, 3 spiked filters with a known number of SARS-CoV-2 RNA molecules.



**Fig. 1.** RT-qPCR log curves of amplification of RdRp gene from extracts of PM10 environmental samples. In blue the ward samples collected 19-20-21/06/2020 respectively; in red the spiked filters.

The **Table 2** reports all the Ct data obtained in replica series performed to confirm these initial results. Moreover, all the amplicons were subjected to a melting curve analysis, high resolution gel electrophoresis and restriction enzyme analysis to confirm reaction specificity (data not shown).

Samples collected on 19/06/20, 20/06/20 and 21/06/20 showed signals in the range 36.7–38.3 cycles, while the samples collected on 22/06/20 and 23/06/20 always appear negative or above the 40 cycle, considered as significant threshold.

### 3.3. Infectivity assay on Vero cells

Partial SARS-CoV-2 RNA has been detected in airborne samples; however, the impact of these traces in terms of potential infectivity is yet not completely known. To this end, we designed a simple model of *in vitro* infectivity evaluation for the detection of the minimal viral quantity (expressed as threshold cycle, after Real Time semi-quantitative PCR) able to infect and replicate in host cells.

We collected twelve samples from swabs of patients attended at the ER of Monfalcone Hospital, whose positivity for SARS-CoV-2 infection was detected by RT QPCR (Allplex SARS-CoV-2 assay, Seegene, Seoul,

**Table 2**

Ct values of RdRp gene amplification of the PM<sub>10</sub> filter RNA extraction from hospital ward (four replicates) and from spiked control filters, both clean/new and used/environmental PM<sub>10</sub>, sampled in 2018; N.D. = not detected.

PM <sub>10</sub> Sample filter	Ct for RdRp replicate 1	Ct for RdRp replicate 2	Ct for RdRp replicate 3	Ct for RdRp replicate 4
June 19, 2020	37.4	36.7	37.7	37.1
June 20, 2020	38.5	N.D.	38.3	N.D.
June 21, 2020	40.3	N.D.	38.3	N.D.
June 22, 2020	40.5	N.D.	N.D.	N.D.
June 23, 2020	N.D.	39.1	N.D.	N.D.
Clean spiked E+6 copies	35.1			
Used spiked E+6 copies	39.1			
Used spiked E+8 copies	37.0	38.4		

South Korea). In **Table 3** we report the threshold cycle of each sample. To note, this kit did not allow the quantification of the viral load, however, it is known that the threshold cycle inversely correlated with the quantity of the virus.

The specimens were subsequently seeded on Vero E6 cells, a permissive, *in vitro* widely accepted, host for SARS-CoV-2 replication. In **Fig. 2** the crystal violet staining performed after 7 days of infection are shown. Crystal violet can be used as a viability assay, it binds DNA and protein of attached cells, mostly indicative of viable cells, meanwhile the dead cells were detached during the procedures (Feoktistova et al., 2016).

The evolution of Ct and viral copy/ml measured at day 0, day 2, day 5 and day 7 in Vero cells inoculated with the 12 samples are reported in **Table 4**, together with the evidence of presence (+) or absence (–) of cytopathic effect, supporting the infectivity assessment of the sample. The original viral quantity used to perform the infection is also showed.

Only the specimens 3, 5, 7, 11 developed an evident cytopathic effect at the day 7<sup>th</sup>, and their viral load increased at the time points investigated (2, 5 and 7 days after virus inoculation). For these samples the viral quantity used for infection is above E+10 viral copies. Instead, the other samples were not able to replicate in this permissive cell culture (less than initial E+10 viral copies).

Therefore, E+10 viral copies (corresponding to Ct < 24) can be considered as a cutoff below which the samples maintained an infectivity potential.

The cytopathic effect could be thus considered unlikely for the environmental PM<sub>10</sub> samples, showing amplification of viral RNA at Ct = 36 or higher.

## 4. Discussion

The airborne transmission of SARS-CoV-2 is still under debate and the main health authorities, although recommending precautions when managing COVID-19 patients especially during procedures able to generate aerosol (e.g. tracheal intubation and dentistry), claim also the need for further research in this field (WHO, 2020; CDC, 2020; ECDC, 2020).

In the present work a short-term air monitoring was conducted at a geriatric COVID-19 healthcare facility in Trieste (Italy), where SARS-CoV-2 RNA traces were detected. Evidence of viral RNA presence reported in **Table 2** is coherent with those of the number of patients in the considered ward: the first day of the monitoring presents the highest number of patients and positive RNA finding in sampled indoor PM<sub>10</sub>.

Previous detection of SARS-CoV-2 RNA (mostly partial viral nucleic acid) have been described in airborne samples; however, the impact of these traces in terms of potential infectivity has not been yet well characterized in a controlled univocal infection model.

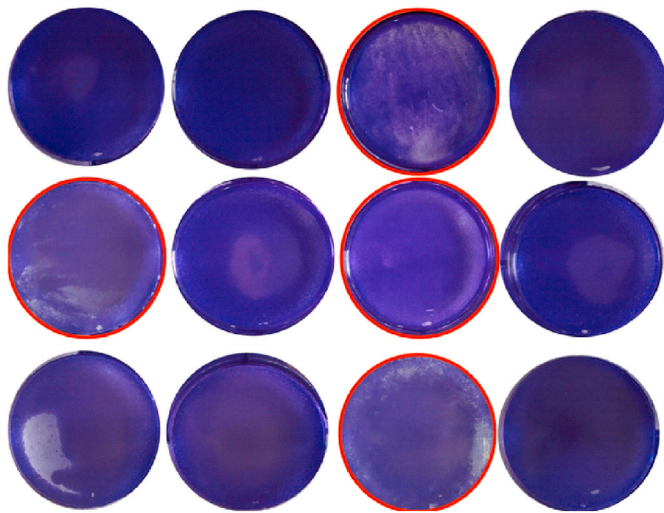
We estimated the potential threshold of infectivity on Vero E6 cells in a set of 12 clinical swab specimens. Our experimental setting showed that E+10 viral copies (approximately equivalent to Ct<24) are able to

**Table 3**

Threshold cycles of the 12 patients enrolled at the ER.

Sample	E gene Ct	RdRp gene Ct	N gene Ct
1	27.7	27.1	26.0
2	28.4	28.1	27.0
3	20.6	20.1	18.5
4	28.9	29.1	27.5
5	25.0	24.4	23.8
6	37.8	N.D.	36.8
7	23.4	25.6	21.3
8	33.6	34.0	32.7
9	35.0	36.2	33.3
10	37.7	36.2	37.0
11	24.7	24.6	22.8
12	N.D.	38.6	N.D.

N.D.: not detected.



**Fig. 2.** Representation of the crystal violet staining of the 12 samples inoculated on Vero E6 cells. First row: patients 1,2,3,4; Second row: patients 5,6,7,8; Third row: patients 9,10,11,12. The darker violet corresponds to the viable cells still attached at the wells (see patients 1,2,4,6,8,9,10,12), instead, where the cells are less viable due to the infection they tend to detach and the staining corresponding to a low number of cells is weak (patients 3,5,7,11, red circled).

generate a cytopathic effect, thus it can be considered as a cutoff below which the samples may maintain infectivity potential at least *in vitro*. In fact, it is technically difficult to predict if the same samples that are able to infect the Vero E6 cells belonged to an individual able to transmit SARS-CoV-2 to other people in a real situation. Nevertheless, our proposed model for infectivity assessment was previously suggested by Wölfel et al., (2020) as a method for early discharge of COVID-19 patients from hospital to home isolation. The authors reported that when the initial viral load was above E+10 viral copies the proportion of positive viral culture was 100% but when the viral load was less than E+6 they never yielded viral amplification *in vitro*. So, they defined on the basis on *in vitro* cell culture that E+5 is the cutoff for patient discharge after 10 days of symptoms.

Other previous studies observed strong correlation between the initial threshold cycle and the success rate of virus amplification in Vero E6 cell culture being below 30% when the Ct was above 30 (Singanayagam et al., 2020; La Scola et al., 2020, Yamada et al., 2021).

In our experiments the threshold for infectivity seems to be lower, however, some difference can be expected due to the different RNA isolation procedures and reagents, moreover, the two studies cited did not report the volumes employed for the extraction, RNA elution and real time PCR experiments that can affect the final results. To date, in the ECDC (European Centre for Disease Prevention and Control) guidelines,

**Table 4**

The cycle threshold (Ct) and the viral load (expressed as viral copies/ml) at day 0, 2, 5 and 7 post SARS-CoV-2 inoculation. The quantity of virus initially used to infect the cells at day 0, as well as the microscopically evaluation of the cytopathic effect at day 7 are reported.

Sample	Day 0		Day 2		Day 5		Day 7		Day 0	Day 7
	Ct	viral copy/ml	Ct	viral copy/ml	Ct	viral copy/ml	Ct	viral copy/ml	Viral copies for infection	cytopathic effect
1	24.4	4.16E+09	28.3	1.67E+08	29.6	3.00E+08	27.2	2.66E+08	2.08E+09	-
2	25.4	1.76E+09	26.0	1.13E+09	29.2	3.78E+08	27.9	1.46E+08	8.78E+08	-
3	18.4	5.81E+11	15.6	5.49E+12	14.2	9.83E+12	13.8	3.21E+13	2.90E+11	+
4	25.4	1.85E+09	28.7	1.21E+08	29.1	4.19E+08	28.0	1.28E+08	9.26E+08	-
5	21.5	4.37E+10	18.4	5.48E+11	13.9	1.27E+13	13.9	2.97E+13	2.18E+10	+
6	37.8	7.31E+04	33.0	3.65E+06	33.5	2.10E+07	28.6	7.75E+07	3.66E+04	-
7	19.2	2.85E+11	16.2	3.32E+12	14.8	6.84E+12	14.4	1.87E+13	1.43E+11	+
8	29.7	5.53E+07	31.8	9.30E+06	35.0	7.24E+06	28.4	9.17E+07	2.76E+07	-
9	34.2	1.31E+06	32.2	7.10E+06	38.2	8.40E+05	30.7	1.27E+07	6.53E+05	-
10	36.0	3.06E+05	36.9	1.48E+05	33.2	2.53E+07	30.3	1.74E+07	1.53E+05	-
11	21.4	4.86E+10	22.1	2.72E+10	14.0	1.11E+13	13.9	3.04E+13	2.43E+10	+
12	33.5	2.34E+06	31.8	9.98E+06	31.4	8.85E+07	28.2	1.13E+08	1,17E+06	-

there is not yet a consensus on the Ct value that can be considered as an infectivity threshold and most of the laboratories and the diagnostic tests WHO approved reported only the positivity or negativity of the samples on the medical report with no assessment of the viral load (ECDC, 2020; WHO, 2020). Our results are coherent with Bullard et al. (2020), that pointed to the need of extending studies on the subject. Furthermore, despite the daily increment of published papers with the keyword SARS-CoV-2 molecular diagnosis, most articles lack to define accurately the study procedures.

In our work, a successful detection of SARS-CoV-2 RNA in environmental air samples was achieved, although at that time we were not able to assess the potential infectivity of these air samples due to the lack of air-sampler able to preserve the virus integrity. The high Ct identified in this analysis, above the Ct threshold of infectivity later evaluated *in vitro* on Vero E6 cells, could suggest very low risk of infection in the monitoring locations at the geriatric unit.

Our results were in agreement with the previous measurements of SARS-CoV-2 RNA in air samples where low viral RNA quantity was commonly reported, as it can be seen in table supplementary 1 (Chia et al., 2020; Guo et al., 2020; Jiang et al., 2020; Lei et al., 2020; Razzini et al., 2020; Santarpia et al. 2020a, 2020b; Zhou et al., 2020, Ding et al., 2021; Feng et al., 2021; Kenarkoohi et al., 2020). However, other studies failed in evidencing SARS-CoV-2 RNA, but considering that air sampling was affected by several variables (e.g. protocol, timing), by the devices employed together with the degradable nature of nucleic acids, these divergences can occur (Comber et al., 2020). It is important to note also that they could represent either real negative findings or false negative data. Studies on the persistence of RT-PCR diagnostic gene fragments on filters in sampling air flows could help in assessing the extent of potential analyte losses and eventual consequent risk underestimation.

The positive findings here reported were also in line with the epidemiologic studies of COVID-19 outbreaks. Interestingly, in different close environmental settings, (e.g. restaurant, choir practise, transport, healthcare facilities) clusters of infections with several COVID-19 cases were reported (Comber et al., 2020).

Nevertheless, when considering the possible environmental transmission of SARS-CoV-2, it is also important to estimate that different variables account to determine its infectious potentiality.

Indeed, the multiple release of the respiratory droplets containing the virus from infected people (e.g. when individuals speak aloud, spit, sneeze or cough) can increase the risk, especially in indoor environments, as well as the high viral load of the affected index cases. Interestingly, it has been reported in a mathematical model of SARS-CoV-2 spreading that a cough from an individual with high viral load could contain 7.44 million viral copies/m<sup>3</sup> (Riediker and Tsai, 2020); moreover, the risk should increase in case of prolonged exposure and poor aerated inner settings.

Indeed, although the previously published articles of SARS-CoV-2

aerosol detection in indoor environments reported positive results, few attempted and achieved to culture the virus and to prove its infectivity. To date, 6 studies reported this type of analysis (Zhou et al., 2020; Binder et al., 2020, Santarpia et al., 2020a,b, Lednicky et al., 2020) and only half of them achieved evidence of the infectivity of the samples collected.

This partial lack of confirmatory functional results could be related to different technical issues, including the lack of optimization and standardization, scarce diffusion of samplers dedicated to preserve viral viability, with high collection efficiency of for nanometric bioaerosol particles in the range of viral dimensions (Lednicky et al., 2016), or the low sensitivity of culture methods undertaken as well as the high Ct (often above 35) of air samples, (Zhang et Duchaine, 2020), that may have led to an underestimation of the potential risk of SARS-CoV-2 airborne transmission. The dimensional aerosol fraction to be sampled is still an issue to focus. Recently an interesting revised model of SARS-CoV-2 airborne dissemination has been proposed by Zhang and Duchaine (2020), in which the authors claimed the virus transmission through potential short range inhalable aerosols that deposit in the upper respiratory airways. These are relatively large particles with short travel distance and suspension time but great probability of containing infectious virions. Moreover, this type of aerosol does not penetrate deep in the respiratory system, so in line with the evidence that the nasal cells, rich in ACE-2 receptor, should be the main portal of access for SARS-CoV-2 initial infection. Another theoretical approach to the identification of the dimensional selection of bioaerosol to prefer - aiming at the identification and cultivation of the coronavirus of interest - is the quanta approach described by (Buonanno et al., 2020) that parametrized dimensional viral emissions from infected person on the base of their respiratory activities, pointing at scenario-dependent infective bioaerosol size distributions. Very limited experimental studies on infective size fractionation have been reported: Lednicky et al. (2021) considered one single sampling by a personal cascade impactor sampler (PCIS), to screen for SARS-CoV-2 in a car driven by a COVID-19 patient being able to cultivate the virus only from the fraction of particles in the 0.25–0.50 µm size range, that is also the hardest to coagulate (too big for Brownian motions) or settle (too small for effective deposition). Remarkably, the viability of viruses once airborne depends on various factors (temperature, relative humidity, aerosol composition, solar radiation), as well as their resistance during sampling that could inactivate them, and moreover, the timing from air collection to sample absorption on Vero cells is crucial.

Therefore, the airborne transmission of SARS-CoV-2 remains still debated and needs more research to unravel the real contribution of this type of virus dissemination in the present pandemic, even if from the early notes on the pandemic WHO recommend precautions with COVID-19 patients during procedures generating aerosol, and both CDC and ECDC recommend airborne precautions during all stages of the care of COVID-19 patients.

## 5. Conclusions

Although the information about the presence of SARS-CoV-2 RNA in environmental samples can be alarming and require to be cautiously disseminated to the public, it is widely admitted that the only positivity of the molecular test is insufficient to draw definitive conclusions about the airborne transmission of SARS-CoV-2; thus, although an “indirect method” as the one presented in the current paper can provide indication about potential infection, it remains mandatory to set up and deploy methods for assessing the infectivity of the air samples collected in indoor environment at risk.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2021.111200>.

## Credit author statement

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