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## Confirmation of SARS-CoV-2 airborne dissemination indoors using “COVID-19 traps”



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### Introduction

A novel human coronavirus named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (also called COVID-19) emerged in Wuhan, China, in late 2019 followed by the rest of the world, causing a pandemic disease with more than 242 million total cases and nearly 5 million total deaths in 226 different countries, at the end of October 2021.<sup>1</sup> Three main actions have been reported to diminish dissemination of SARS-CoV-2: social distancing of at least 1.5, the use of protective mask and hand washing.<sup>2–4</sup>

However, the routes of SARS-CoV-2 transmission are not still completely understood. In the beginning of the pandemic state, the contact route was supposed to be the main route of transmission followed by large droplets transmission; whereas the aerosol transmission was nearly discarded by the World Health Organization (WHO). Nevertheless, updates related to SARS-CoV-2 in the USA,

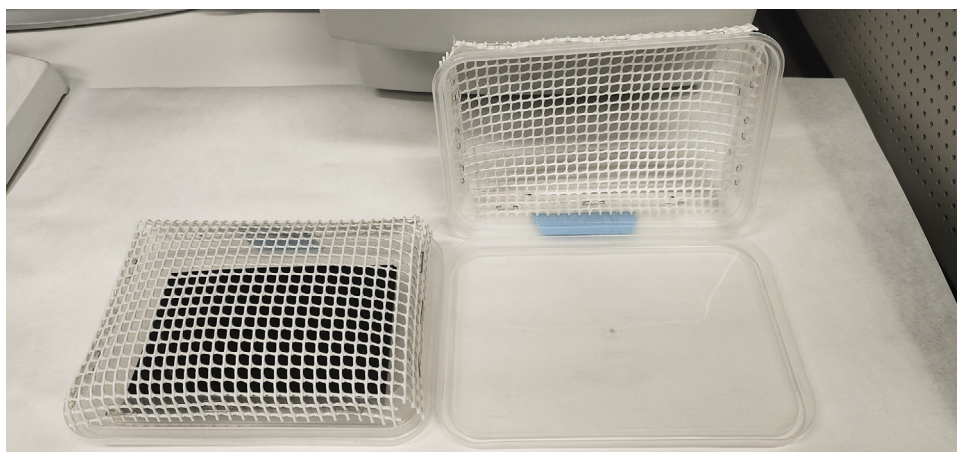
Centers for Disease Control and Prevention (May 7, 2021), and the WHO (April 30, 2021) have found that aerosol transmission could be the main route of SARS-CoV-2 dissemination (57%), followed by large droplet inhalation (35%) and contact route had the lower probability of transmission with only 8%.<sup>5</sup>

Importantly, despite robust efforts, patients and staff acquire SARS-CoV-2 infection in hospitals and this nosocomial infection occurred in rapid super-spreading events when mixing COVID-19 and non-COVID-19 patients through a limited number of highly infectious individuals.<sup>6</sup> Interestingly, as previously hypothesized, despite vaccines administration, SARS-CoV-2 transmission has not been reduced in several countries.<sup>7</sup> In the United States, COVID-19 cases and deaths clearly declined since their peak in early January 2021, due in part to vaccination. However, in July 2021, COVID-19 cases, hospitalizations and deaths increased dramatically, driven by the highly transmissible B.1.617.2 (Delta) variant.<sup>8</sup> In an attempt to confirm the importance of COVID-19 aerosol transmission, we previously developed “COVID-19 traps” to measure the capacity of SARS-CoV-2 aerosol dissemination.<sup>9</sup> Different surfaces were incorporated into these “COVID-19 traps” to analyze the stability of SARS-CoV-2 and the time necessary to detect its presence on these surfaces by RT-PCR. In addition, these surfaces could not be touched by patients or healthcare personnel but was in contact with air at all times. Thus, the aim of this study was to confirm

*Abbreviations:* CWU, COVID-19 ward unit; E, Envelope; HVAC, Heating, ventilation and air-conditioning; N, Nucleocapsid; PP, Polypropylene; RdRP, RNA dependent-RNA polymerase; RT-PCR, Reverse transcription-polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; VTM, Viral transport medium.

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**Fig. 1.** Image of two “COVID-19 traps”. The left one has a surface inside that cannot be touched; whereas the right one is empty and opened. This surface was later tested with nylon swabs moving them horizontally, vertically and transversely across all the sampling area. Immediately, the swab was immersed in VTM and stored at  $-80^{\circ}\text{C}$  until analyzed.

aerosol dissemination from patients with coronavirus infection using “COVID-19 traps”.

## Material and methods

### Patient rooms

In this novel study, “COVID-19 traps” were placed in 20 rooms of patients with a confirmed positive diagnostic in a COVID-19 ward unit (CWU). All the windows remained closed. The rooms where COVID-19 patients were isolated had a ventilation rate of  $1800\text{ m}^3/\text{h}$ . This means that the air of the room was completely renovated 50–60 times per hour. Importantly, the air came 100% from the outside of the hospital. We placed “COVID-19 traps” at 1.5 m of height and 2 m of distance from patients. The use of face masks by healthcare workers and patients were mandatory.

### Surfaces

Our data consisted on 3 different surfaces of  $100\text{ cm}^2$  trapped in boxes with plastic, protective grids to avoid that samples could be touched by the patient or by the healthcare personnel (Fig. 1). The different surfaces were: polypropylene (PP), glass and methacrylate. PP surfaces were obtained from PP black panels and had a semi-gloss finish with a thickness of 2 mm. These PP surfaces had a one-side plastic cover to be removed prior to use, ensuring a clean/non-manipulated surface. Glass surfaces had polished edges and were manufactured according to UNE-EN ISO 1514. They had 4 mm of thickness. Methacrylate surfaces were totally colorless and transparent, with a thickness of 4 mm and obtained from Plexiglas® XT. These materials were selected to be included in the “COVID-19 traps” as two of them gave positive results in our previous study (PP and glass),<sup>9</sup> and the other one (methacrylate) is a material widely used in hospitals (protective masks, partitions...).

The three surfaces included in the “COVID-19 traps” were placed in 20 rooms of patients with confirmed SARS-CoV-2 infection. Surface samples were collected at three different time points (24, 48 and 72H) using nylon swabs immersed in viral transport medium (VTM) (UTM-Copan®) before sampling. Samples were taken moving the swab horizontally, vertically and transversely across the sampling area. The swab was immediately placed into 1 ml of VTM and stored at  $-80^{\circ}\text{C}$  until analyzed.

To correlate with patients’ viral load, nasopharyngeal samples were extracted the day when “COVID-19 traps” were placed in

their rooms. Additionally, samples from patients were extracted using the same nylon swabs immersed in VTM than the ones used for surfaces (UTM-Copan®).

### RNA extraction

Patients and surfaces samples were treated equally during RNA extraction and RT-PCR technique. RNA extraction was performed using the automatized system Nuclisens Easymag® (bioMérieux) based on the ability of silica to bind DNA and RNA in high salt concentrations (Boom technology) as previously explained.<sup>9</sup> In brief, during incubation of the lysed samples, all the target nucleic acids were captured by silica magnetic particles. This way, the Nuclisens Easymag® magnetic device attracts all the magnetic silica, enabling the system to purify the nucleic acids through several washing steps. Then, samples were heated, thus releasing the nucleic acids from silica. Finally, the magnetic silica particles were separated from the nucleic acids by a magnetic device and samples were eluted in  $50\ \mu\text{L}$  of elution buffer.

### RT-PCR

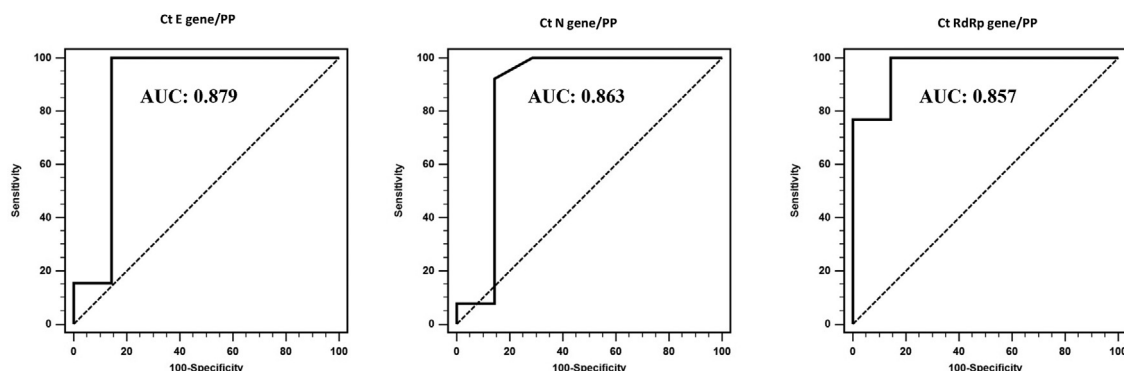
The purified RNA was subjected to amplification by RT-PCR (Allplex™ 2019-nCoV Assay®, Seegene). The CFX96 (Biorad®) platform was used for the amplification process.

Allplex™ 2019-nCoV Assay is a multiplex RT-PCR assay for simultaneous detection of 3 target genes of SARS-CoV-2 in a single tube. The assay is designed to detect RdRP (RNA dependent-RNA polymerase) and N (Nucleocapsid) genes specific for SARS-CoV-2, and E (Envelope) gene for all of Sarbecovirus including SARS-CoV-2. The results were analyzed using the software Seegene Viewer V3.18.005.003.

The Allplex™ 2019-nCoV Assay includes a full process internal control which is composed of MS2 phage genome. This internal control material verifies all steps of the analysis process, including sample extraction, reverse transcription, and PCR to demonstrate proper specimen processing and test validity of each specimen.

### Statistical analyses

Continuous variables were tested for normal distribution by the Kolmogorov-Smirnov test. Comparisons of the groups for continuous variables were performed with the unpaired *t*-test for independent samples or the Mann-Whitney U-Test (as appropriate).



**Fig. 2.** Receiver operating characteristic (ROC) curves for the three genes analyzed in this study in the PP surface. Area under the curve (AUC) for the three genes: (A). AUC for Ct E was 0.879 with  $p = 0.0019$ ; (B). AUC for Ct N was 0.863 with  $p < 0.0001$  and (C). AUC for RdRp was 0.857 with  $p = 0.0059$ .

To compare different predictive values, areas under the receiver-operating characteristic curves were constructed for sensitivity and specificity values. The Youden index was used to determine the relationship between the three different surfaces and the three genes analyzed with the best combination of sensitivity and specificity in order to establish a cut-off value. All  $p$  values  $< 0.05$  were accepted as statistically significant. The statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS), version 21.0 for Windows software program (Chicago, IL, USA).

## Results

As observed in Table 1, in total, 56 positives out of 180 analysis were found in three different surfaces (PP, glass and methacrylate) at 24, 48 and 72 h. This means that more than 30% of samples analyzed were positive for COVID-19 airborne dissemination. Interestingly, in our previous study, positives were only found at 72 h, and we explained this fact assuming that an accumulation of virus in time was necessary in the surfaces to be detected by RT-PCR. Remarkably, in our first study, patients' viral load was significantly lower than in this one.

Despite the fact that the air of the room was completely renewed every minute, many positives were found in the surfaces trapped in the "COVID-19 traps", thus showing the dissemination capacity of SARS-CoV-2 virus on fomites (Table 1).

Moreover, and as observed in previous studies, 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.<sup>10</sup> In this novel study, positives were found in several rooms and in all surfaces at 24, 48 and 72 h. As can be observed in Table 1, in general, it could be said that patients with higher viral load (Ct from 10 to 19) showed positive results regardless the nature of the surface and the exposition time. Unlikely, patients with lower viral load (Ct above 20) showed negative results or the number of positive surfaces decreased significantly, and only after 48 or 72 h some positives were found. These observations highlight the importance of patients' viral load in the SARS-CoV-2 dissemination. To confirm these observations, statistical analyses were performed and the optimal cut-off for the three genes and the three surfaces analyzed in this study were obtained (Table 2). As observed in Table 2, the PP surface has the best sensitivity, specificity and area under the curve (AUC) for all genes (Fig. 2), with a very low significance level, thus showing the robustness of our analyses.

## Discussion

Our work shows that viruses are released in microdroplets small enough to remain aloft in the air and pose a risk of exposure at distances 2 m or beyond from an infected individual. Res-

piratory viruses can be transmitted in three different ways. First, contact transmission with an infected person or touching a surface that has been previously contaminated. Secondly, through large of small respiratory droplet transmission containing the virus near an infected person. Third, through airborne transmission of smaller droplets and particles (aerosols) suspended in the air. This aerosol transmission can infect longer distances and time than droplet transmission;<sup>11</sup> thus, there is significant potential for inhalation exposure to viruses in respiratory microdroplets at short to medium distances.<sup>12</sup>

Heating, ventilation and air-conditioning (HVAC) have a pivotal role in determining airborne diseases. In our study, despite having a ventilation rate of at least 50 times per hour, and being much more than the recommended by leading organizations/societies for HVAC system in the management of COVID-19 patients;<sup>13</sup> we could detect SARS-CoV-2 in more than 30% of samples analyzed. Therefore, it seems to be necessary to adjust the thresholds and measures to provide sufficient and effective ventilation.

During the initial stages of the pandemic, surface transmission was thought to be one of the most important routes of dissemination; however, latest research suggests that although SARS-CoV-2 can persist for days on inanimate surfaces, attempts to culture the virus from these surfaces were unsuccessful, thus confirming that this transmission route was not as important as previously hypothesized.<sup>14</sup> In this context, there are several studies with contradictory results about the dissemination and infection capacity of SARS-CoV-2 from different surfaces. In one of them, air and surface samples were analyzed in 13 individual with COVID-19 and viral contamination was found in all samples tested and even in air samples. Additionally, the authors support the idea of a strong influence of airborne dissemination as patients were generally less mobile.<sup>15</sup> Similarly, in another study from three symptomatic patients, positives were found in almost all surfaces in their rooms; however, all air samples were negative, and authors confirm that surfaces could be a potential medium of transmission.<sup>16</sup> Importantly, stability and transmission of coronaviruses have been also studied in laboratory conditions.<sup>10,17,18</sup> In one of these studies, it was confirmed that human coronaviruses can remain infectious on surfaces for up to 9 days at room temperature. Moreover, the droplets present in the form of an aerosol of an infected patient can not only easily spread, but also easily settle and last for several hours on a surface.<sup>17</sup> These results were in consonance with a previous study, analyzing coronaviruses' persistence on metal and non-metal samples.<sup>18</sup> It was found that coronaviruses' persistence was at least 5 days at 21 C in different surfaces; whereas it could be rapidly inactivated by brass and copper nickel surfaces in less than 60 min. Additionally, more recent studies analyzing the stability and infectivity of SARS-CoV-2 on inert surfaces have been con-

**Table 1**  
RT-PCR Ct cycle of 3 different genes associated to COVID-19 detection at 3 different times.

Patient	Ct gene E Patient	Ct gene RdRp	Ct gene N	Time	Material	Ct gene E Surface	Ct gene RdRp	Ct gene N	O2 support
Pat 1	11.4	11.9	13.2	24h	PP	Neg	Neg	Neg	Nasal Cannula
				48h		Neg	Neg	Neg	
				72h		38.3	39.1	37.2	
				24h	Glass	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		37.7	37.5	38.2	
Pat 2	10.2	10.5	10.9	24h	PP	35.2	36.2	34.5	No
				48h		35.7	36.6	36.4	
				72h		38.1	37.5	38.2	
				24h	Glass	30.7	32.2	29.1	
				48h		36.8	36.9	35.1	
				72h		37.8	37.3	38.2	
				24h	Metachrylate	39.2	38.7	36.3	
				48h		36.1	39.2	35.8	
				72h		Neg	Neg	Neg	
Pat 3	16.1	16.5	16.3	24h	PP	Neg	Neg	Neg	BiPAP
				48h		36.9	39.3	35.6	
				72h		36.6	38.9	37.9	
				24h	Glass	Neg	Neg	Neg	
				48h		33.1	34.2	32.4	
				72h		35.9	38.3	36.8	
				24h	Metachrylate	37.8	38.8	35.5	
				48h		37.5	38.2	37.7	
				72h		38.6	39.1	37.2	
Pat 4	14.6	15.3	14.8	24h	PP	36.9	37.2	36.5	Nasal Cannula
				48h		38.6	39.1	39.2	
				72h		34.6	35.4	33.1	
				24h	Glass	Neg	Neg	Neg	
				48h		38.3	38.6	37.1	
				72h		35.9	35.7	33.8	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		34.1	35.4	33.1	
Pat 5	15.9	16.7	14.1	24h	PP	Neg	Neg	Neg	Nasal Cannula
				48h		Neg	Neg	Neg	
				72h		39.0	38.8	37.9	
				24h	Glass	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		35.2	36.6	36.2	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		37.0	38.1	37.9	
				72h		38.9	39.5	37.6	
Pat 6	18.5	18.7	17.1	24h	PP	38.4	36.2	37.6	Nasal Cannula
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Glass	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		39.1	37.2	38.9	
Pat 7	12.9	14.8	12.0	24h	PP	36.9	37.5	38.8	Nasal Cannula
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Glass	39.1	38.2	38.4	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
Pat 8	19.5	19.3	19.9	24h	PP	Neg	Neg	Neg	Nasal Cannula
				48h		38.6	39.6	37.8	
				72h		Neg	Neg	Neg	
				24h	Glass	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		38.1	39.5	39.2	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	

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**Table 1** (continued)

Patient	Ct gene E Patient	Ct gene RdRp	Ct gene N	Time	Material	Ct gene E Surface	Ct gene RdRp	Ct gene N	O2 support
Pat 9	16.2	16.9	17.1	24h	PP	Neg	Neg	Neg	Nasal Cannula
				48h		Neg	Neg	Neg	
				72h		34.1	36.2	33.7	
				24h	Glass	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		36.9	35.3	35.8	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		37.5	38.2	36.1	
Pat 10	21.5	20.8	17.9	24h	PP	Neg	Neg	Neg	Nasal Cannula
				48h		37.8	38.6	37.2	
				72h		Neg	Neg	Neg	
				24h	Glass	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Metachrylate	37.9	39.1	38.9	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
Pat 11	22.3	21.8	24.1	24h	PP	Neg	Neg	Neg	No
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Glass	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
Pat 12	17.6	17.8	17.1	24h	PP	Neg	Neg	Neg	HEPA Filter 5L
				48h		38.3	38.8	38.5	
				72h		Neg	Neg	Neg	
				24h	Glass	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
Pat 13	24.4	25.2	24.9	24h	PP	Neg	Neg	Neg	No
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Glass	37.9	36.9	38.1	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Metachrylate	39.1	38.6	37.9	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
Pat 14	22.9	24.9	21.8	24h	PP	Neg	Neg	Neg	Nasal Cannula 3L
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Glass	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
Pat 15	20.4	21.3	20.9	24h	PP	Neg	Neg	Neg	CEPAP
				48h		37.9	38.9	36.7	
				72h		Neg	Neg	Neg	
				24h	Glass	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		37.8	39.1	38.1	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
Pat 16	26.9	27.3	26.5	24h	PP	Neg	Neg	Neg	No
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Glass	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	

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**Table 1** (continued)

Patient	Ct gene E Patient	Ct gene RdRp	Ct gene N	Time	Material	Ct gene E Surface	Ct gene RdRp	Ct gene N	O2 support
Pat 17	16.7	15.9	17.1	24h	PP	Neg	Neg	Neg	Nasal Cannula 4L
				48h		37.9	39.4	38.1	
				72h		Neg	Neg	Neg	
				24h	Glass	Neg	Neg	Neg	
				48h		38.1	39.6	38.7	
				72h		37.4	36.9	38.5	
				24h	Metachrylate	37.9	38.5	37.3	
				48h		39.1	39.0	38.3	
				72h		38.2	37.6	39.2	
Pat 18	17.1	18.2	17.5	24h	PP	38.4	37.8	39.5	No
				48h		Neg	Neg	Neg	
				72h		36.9	37.3	38.2	
				24h	Glass	37.9	39.3	38.6	
				48h		Neg	Neg	Neg	
				72h		36.9	37.2	38.1	
				24h	Metachrylate	35.6	36.5	36.4	
				48h		Neg	Neg	Neg	
				72h		37.2	38.6	36.9	
Pat 19	22.1	21.4	20.9	24h	PP	Neg	Neg	Neg	No
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Glass	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		38.5	38.1	38.5	
Pat 20	24.5	26.9	22.8	24h	PP	Neg	Neg	Neg	No
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Glass	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	
				24h	Metachrylate	Neg	Neg	Neg	
				48h		Neg	Neg	Neg	
				72h		Neg	Neg	Neg	

Abbreviations: CWU: COVID19 ward unit; E: Envelope; N: Nucleocapsid; PP: polypropylene; RdRp: RNA dependent-RNA polymerase.

**Table 2**  
Data obtained from ROC curves.

Gene/Surface	Cut-off	Sensitivity	Specificity	Significance level (p)	AUC
Ct E/PP	21.5	100.00	85.71	0.0019	0.879
Ct E/Glass	17.1	72.73	88.89	0.0093	0.798
Ct E/Methacrylate	17.1	66.67	87.50	0.0212	0.771
Ct N/PP	19.9	92.31	85.71	0.0059	0.863
Ct N/Glass	17.5	72.73	66.67	0.05	0.732
CT N/Methacrylate	17.9	83.33	75.00	0.0392	0.760
Ct RdRp/PP	21.3	100.00	85.71	<0.0001	0.857
Ct RdRp/Glass	19.3	81.82	66.67	0.03	0.758
Ct RdRp/Methacrylate	18.7	75.00	87.50	0.0001	0.844

Abbreviations: AUC: Area under the curve; E: Envelope; N: Nucleocapsid; PP: polypropylene; RdRp: RNA dependent-RNA polymerase.

ducted.<sup>19</sup> All of them agree with the fact that SARS-CoV-2 can last on different surfaces for times ranging from hours to a few days but a rapid inactivation is possible by using commonly available chemicals and biocides on surfaces; thus, washing hands and regular disinfection practices should reduce the possibilities of transmission of the coronavirus by this potential route of infection.

In contrast, in the beginning of the pandemic state, it was thought that airborne transmission of SARS-CoV-2 was unlikely, but growing evidence has highlighted that infective microdroplets are small enough to remain suspended in the air and expose individuals at distances beyond 2 m from an infected person.<sup>20</sup> Importantly, in July 2020, over 200 scientists published a statement calling for international organizations to recognize the potential for airborne spread of COVID-19 as they were concerned that people

would not be fully protected by adhering to the current recommendations.

The evidence for the potential airborne transmission of SARS-CoV-2 via aerosols was analyzed in a systematic review where 16 air sampling studies were included.<sup>21</sup> Detection of SARS-CoV-2 RNA was reported in 12.5%–66.7% of air samples and the number of samples varies between 3 and 40 samples (the higher percentage (66.7%) was found in a study with only 3 samples analyzed).<sup>22</sup> It is important to remark that in our study, 180 samples were analyzed and 56 of them resulted positive (31.1%) being, as far as we know, one of the studies with the highest number of samples and with higher percentage of positives. In addition, the use of our “COVID-19 traps” is an easy method that avoids the use of pumps or expensive bio samplers.

The presence of SARS-CoV-2 was previously tested in 6 different surfaces in our pilot study.<sup>9</sup> It was found in two different surfaces (PP and glass) at 72 h in the room of a patient with a nasal cannula. For that reason, in our novel study, we decided to include these two surfaces in our “COVID-19 traps” and add methacrylate, as this is a barrier surface widely used in hospitals. Interestingly, we found positives in all the surfaces tested and at all times (24, 48 and 72 h). These observations highlight that the most important factor associated to SARS-CoV-2 airborne dissemination is patients’ viral load, as in the rooms of patients with high viral load, positives were found in all surfaces and at all times; whereas in the rooms of patients with low viral load, positives were only found at 48 or 72 h, thus confirming the hypothesis of our pilot study stating that it was necessary an accumulation of the virus in time when patients’ viral load was low to be detected using the RT-PCR technique. Furthermore, statistical analyses carried out in this study confirm these observations.

In addition, in our pilot study, methacrylate was not positive for SARS-CoV-2 detection; however, we decided to include in this novel analysis. Remarkably, positives were found with the same frequency in all surfaces (Table 1), so the stability of SARS-CoV-2 in methacrylate was the same than the one observed in PP or in glass. These observations have been confirmed with the statistical analyses performed in this study (Table 2). This result should be taken cautiously and further studies should be performed to confirm this observation; however it could be interesting to limit the use of this material in hospitals. To sum up, our results point the importance of coronavirus airborne dissemination and accumulation indoors and may provide clues to understand aerosol transmission of SARS-CoV-2.

### Limitations, conclusions and future perspectives

This study has limitations. Firstly, most of patients were caucasians, and the data should not be extrapolated to other ethnic groups. Secondly, the detection of the virus in the air through PCR assays merely indicates presence and does not provide information regarding viability or infection risk. However, many studies indicate that viral culture is surprisingly difficult, being a reason why virus isolation in cell culture is much less sensitive than detection by molecular methods.<sup>23,24</sup> This way, finding viral RNA in air samples should be interpreted as more likely to indicate the presence of live virus than not, as per the precautionary principle, should always reinforce effective infection control.<sup>25</sup> In addition, we cannot confirm that all positives obtained were by airborne transmission as some particles could appear by cleaning activities or skin flakes and fibers. However, as can be observed in the data obtained, positives were found just 24 h before placing the COVID-19 traps, especially in patients with higher viral load. In addition, many patients were in bed and could not move, so bedding and cleaning activities were also significantly reduced in their rooms. Moreover, COVID-19 traps were designed to avoid direct contact and to limit the entrance of fibers or other materials into the trapped surfaces. Furthermore, as can be easily imagine, all sanitary personnel wore sterilized material when entering the room, so contamination via sanitary personnel should be also excluded or very limited. Additionally, traps were placed more than 2 m from the patients and above patient beds; thus, this type of contamination, although can be produced, should be very restricted with our experimental design. For all these reasons we believe that most of positives were by airborne transmission and not by large droplets.

All these data support the recommendation of social distancing, the use of mask and to provide sufficient and effective ventilation due to airborne dissemination to avoid COVID-19 transmission indoors and especially in hospitals. Moreover, the use of methacrylate in the hospitality environment is no guarantee for safety in

relation to SARS-CoV-2, as its stability on this material is high. In addition, as this is a cheap and easy to perform method for COVID-19 detection, these “COVID-19 traps” could be used in public areas such as schoolrooms, courthouses, police offices, hospital waiting rooms, theatres or cinemas to rapidly detect dissemination and potential new outbreaks of this mortal disease.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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