SUPPLEMENTAL MATERIALS

Appendix 1. Indoor air quality device specifications

The IAQ monitoring device is composed of few sensors which are described below:

- Temperature and relative humidity are accessed by a capacitive digital sensor;
- Carbon dioxide (CO_2) concentration is measured by a dual-channel (reference + measuring channel) near
infrared dispersion principle;
- Total volatile organic compounds (TVOC) is measured by a metal-oxide digital sensor with artificial bee colony algorithm;
- Particulate matter (PM) is quantified by a laser scattering particle counter.

Parameter	Range	Accuracy
Temperature (ºC)	-40 ~100	±0,2
Relative Humidity (%)	0 ~100	±3,5
CO_2 (ppm)	400 ~ 10.000	±(30 + 3% measured vale)
TVOC (ppb)	0 ~1.187	N/A
PM (µg/m³)	0 ~1.000	±10µg at < 100 µg/m³ ±10% at > 100 µg/m³

Appendix 2. Molecular analysis

Total RNA extraction

Total ribonucleic acid (RNA) from PTFE membrane sample was extracted using acid guanidiniumphenol based reagent (Trizol® - Thermo Fisher Scientific - Waltham, Massachusetts, USA). The PTFE membrane was immersed in 1 mL of Dulbecco's Modified Eagle's medium (DMEM) and homogenized. 300 µl of DMEM containing PTFE material was added to 0.7 ml of Trizol, followed by the addition of 200 µl of chloroform. Then the 1.5 mL tube was incubated for 5 minutes and centrifuged for 5 minutes at 12,000×g. The aqueous layer (which contains RNA) was transferred to a new tube and 600 uL of isopropanol (Merck – Darmstadt, Germany) was added. After mixing, the

sample was incubated for 30 minutes. Then, the sample was centrifuged for 15 minutes at 12,000×g at 4°C and the supernatant was discarded. The remaining pellet was resuspended in 500 uL of 70% ethanol and centrifuged for another 5 minutes at 12,000×g at 4°C. The supernatant was discarded, and the RNA pellet was air dried for 15 minutes. To solubilize the RNA pellet, it was resuspended in 40 uL of DNAse/RNase-free water (Merck – Darmstadt, Germany) and incubated at 40°C for 10 minutes.

Primer and Probe sets specific for SARS-CoV-2

Primer and Probe sets were taken directly from the primer sets listed by the Centers of Disease Control (CDC). The forward/reverse primers and probes were synthesized by Thermo Fisher Scientific (Waltham, Massachusetts, USA):

- N1_F 5'-GACCCCAAAATCAGCGAAAT-3'
- N1_R 5'-TCTGGTTACTGCCAGTTGAATCTG-3'
- N1_P 5'-FAM-ACCCCGCATTACGTTTGGTGGACC-QSY7-3'
- N3_F 5'-GGGAGCCTTGAATACACCAAAA-3'
- N3_R 5'-TGTAGCACGATTGCAGCATTG-3'
- N3_P 5'-VIC-CACATTGGCACCCGCAATCCTG-QSY7-3'
- RNAseP_F 5'-AGATTTGGACCTGCGAGCG-3'
- RNAseP_R 5'-GAGCGGCTGTCTCCACAAGT-3'
- RNAseP_P 5'-JUN-TTCTGACCTGAAGGCTCTGCGCG-QSY7-3'

Synthetic DNA and Total RNA SARS-CoV-2 positive controls

In order to quantify the number of viral gene copies in each sample from the measured cycle threshold (Ct) values, a standard curve was developed using synthetic DNA designed specifically for a region of gene N of SARS-CoV-2. The specific region for amplification of N1 primers was taken directly from NCBI database (Accession Number LC528233). The synthetic DNA of SARS-CoV-2 N1 region template was synthesized by Macrogen (Seoul, South Korea) (N1_Template 5'-

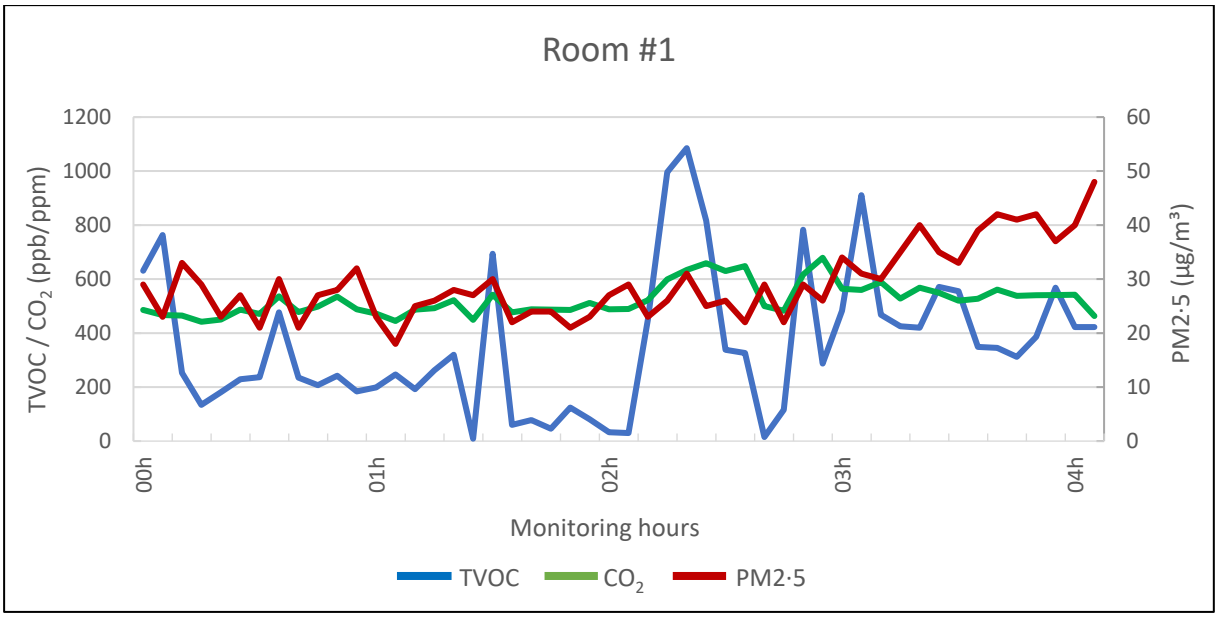
GACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAA CTGGCAGTAACCAGA-3'). An 8-log standard curve was run in duplicate beginning at a concentration of 1 nM. Also, a positive control containing SARS-CoV-2 viral RNA was prepared.

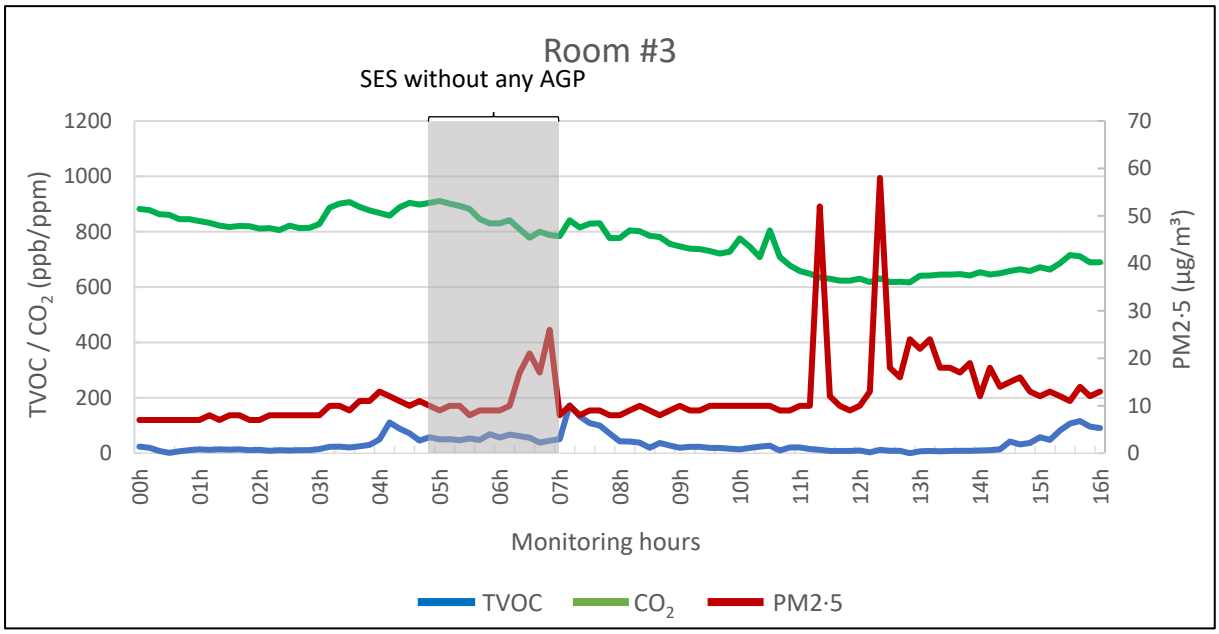
Quantitative RT-PCR

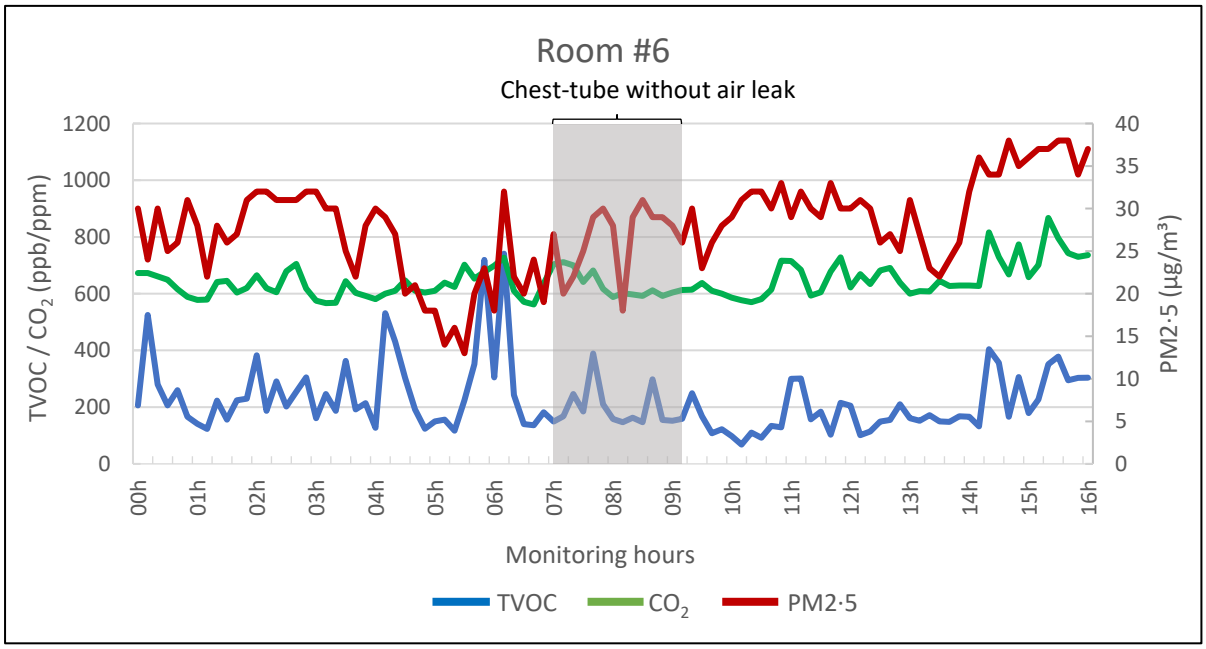
Quantitative RT-PCR was carried out using SuperScript III One-Step RT-PCR System with Platinum Taq DNA-Polymerase (Thermo Fisher Scientific - Waltham, Massachusetts, USA). Each RT-PCR reaction consisted of a total volume of 20 ul containing 1 uL of 10 uM forward/reverse primers and 5 uM of specific probe, 5 uL of Total RNA, 10 uL of 2X Reaction Mix, 0.8 uL Enzyme Mix and 3.2 uL of DNAse/RNase-free water (Merck – Kenilworth, USA). All samples were done in duplicate and each RT-qPCR run included a positive and a negative control. Quantitative positive results were considered for Ct values lower than 38.00 for the SARS-CoV-2 marker N1. RT-qPCR runs were performed with CFX96 Real-Time PCR Detection System (BioRad – Hercules, USA) for 10 minutes at 50°C, 5 minutes at 95°C and then 40 cycles at 95°C for 10 seconds and another 30 seconds at 60°C. Threshold cycle (Ct) value was calculated using CFX Manager Software (BioRad – Hercules, USA). Qualitative RT-PCR reactions were performed as multiplex reactions containing all primers and probes sets from CDC (N1, N3 and RNAseP). All other reactions conditions and parameters followed the same conditions described above for Quantitative RT-PCR.

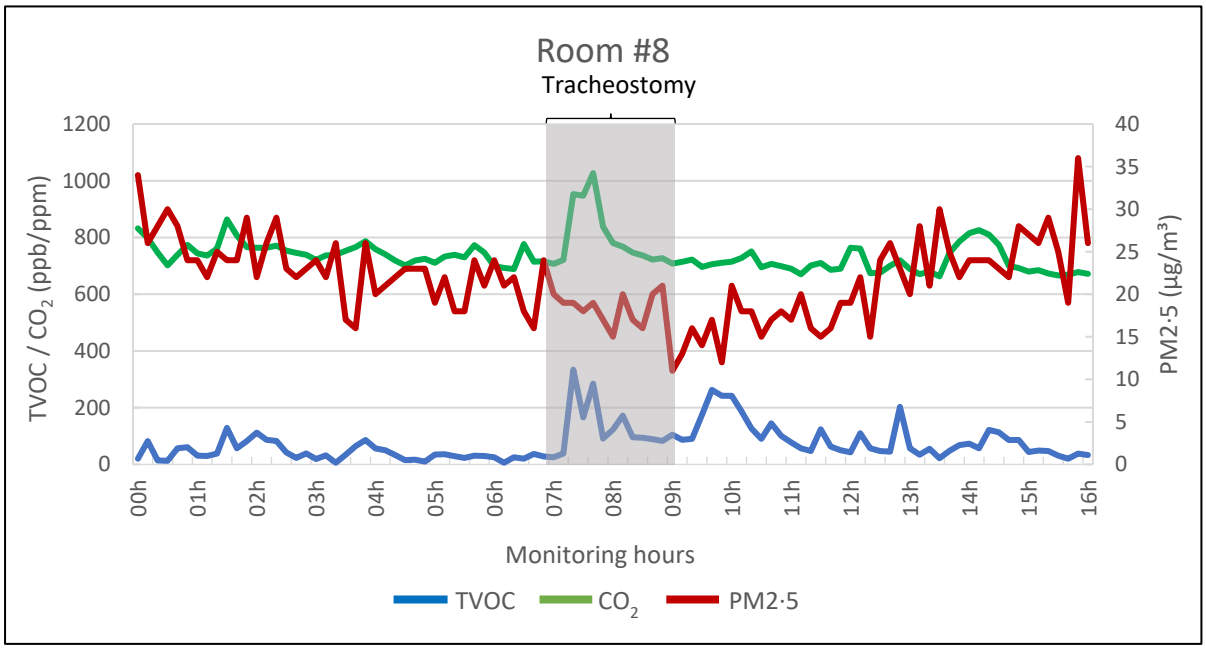
Appendix 3. Charts with IAQ timeline

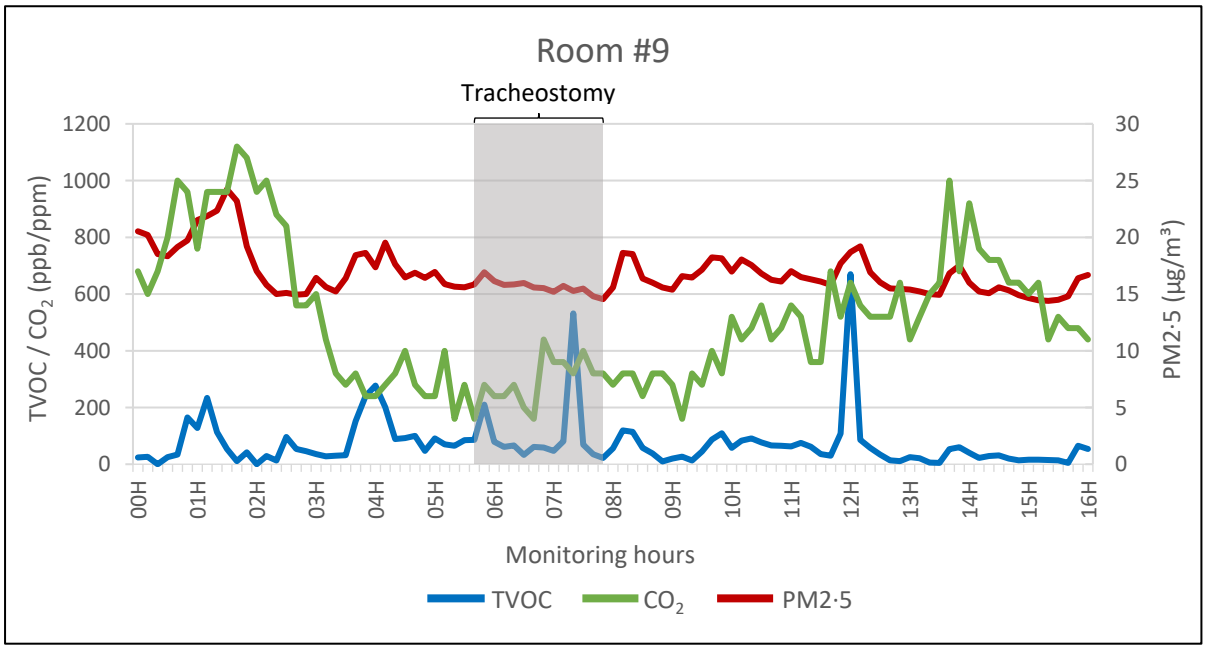
Charts showing the timeline of TVOC, CO2 and PM2·5 levels in rooms #1, #3, #6, #8, and #9 during each monitoring are available below. Grey boxes represent the period in which short-exposure samples were collected and/or AGPs were performed.
IAQ sensor was accidentally turned off in rooms #1 and #4 and, consequently, their timelines are shorter and not available respectively. In room #1, the short exposure sample was collected after the device was turned off and, hence, it is not shown on the timeline.


Room #1
TVOC / CO_2 (ppb/ppm)
PM2·5 (µg/m³)
Monitoring hours
TVOC
CO_2
PM2·5


Room #3
SES without any AGP
TVOC / CO_2 (ppb/ppm)
PM2·5 (µg/m³)
Monitoring hours
TVOC
CO_2
PM2·5


Room #6
Chest-tube without air leak
TVOC / CO_2 (ppb/ppm)
PM2·5 (µg/m³)
Monitoring hours
TVOC
CO_2
PM2·5





SES: short-exposure sample; AGP: aerosol-generating procedure; TVOC: total organic volatile compounds; CO2: carbon dioxide; PM2.5: particulate matter < 2.5µm