

## **Supplementary Information**

### **The SARS-CoV-2 viral load in COVID-19 patients is lower on face mask filters than on nasopharyngeal swabs**

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## Supplementary Information

### Aerosol generating system in the laboratory

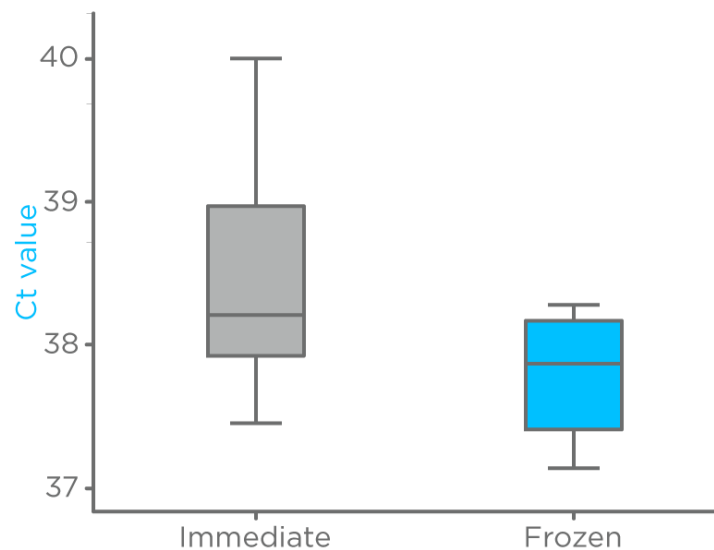


**Supplementary Figure 1. Laboratory set up of the particle aerosolization system used for dispersing virus onto filters.**

## Effect of immediate RNA extraction from filters or after frozen storage

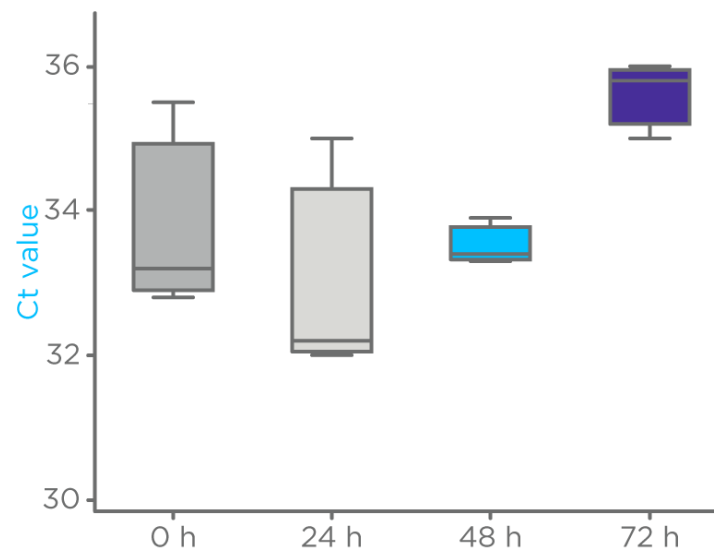
To investigate whether a limited duration freezing and thawing of filters prior viral RNA extraction had significant effect on the outcome, 500 copies/ $\mu\text{L}$  of inactivated SARS-CoV-2 were aerosolized and captured on filters (n=10). After aerosolization, the filters were divided into two groups. One group of filters (n=5) was immediately treated with lysis buffer for viral RNA extraction, while the remaining five filters were stored frozen for 5 days before extracting viral copies from the filters.

Quantitative-RT-PCR testing of filters stored in a dry, frozen state did not yield statistically significance differences (p-value of 0.7) to filters extracted immediately following aerosolization of inactivated SARS-CoV-2 (Supplementary Figure 2).



**Supplementary Figure 2. Storage of filters in the dry, frozen state did not yield different results from filters extracted immediately following aerosolization of inactivated SARS-CoV-2.**

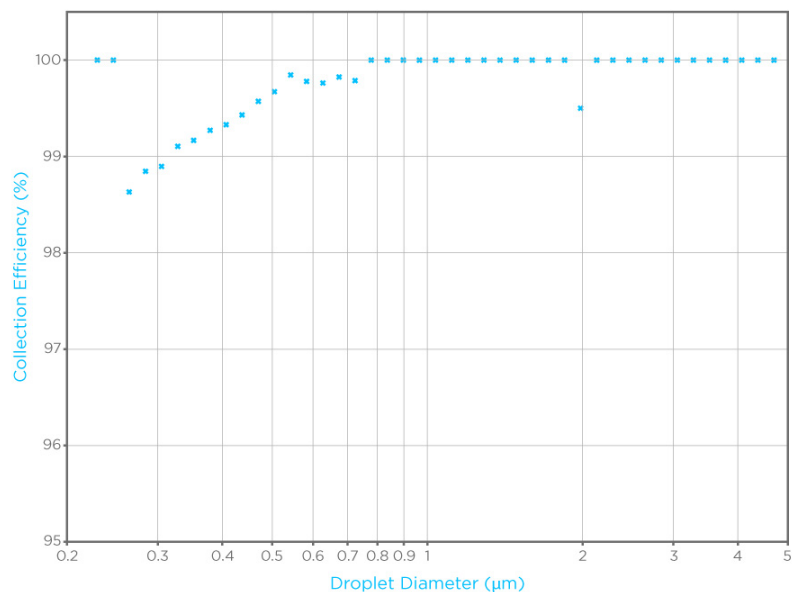
## Inactivated SARS-CoV-2 was stable at room temperature for up to 48h on dry filters that were not exposed to breath



**Supplementary Figure 3. SARS-CoV-2 was stable on filters for up to 48h.** Filters not exposed to breath first showed stable Ct values for 48h which then increased at 72h. Wilcoxon rank sum testing between time point 0 and time 24h, 48h and 72h for filters not pre-exposed to breath (n=3) led to p-values to 0.3, 0.1 and 0.05, respectively. Results from filters that were first exposed to human breath before being spiked with inactivated SARS-CoV-2 are shown in Figure 4 in main manuscript.

## Filter particle collection efficiency determination

The electrostatic filter collection efficiency was tested by measuring the aerosol particle distribution and concentration with and without the filter in place for 5 minutes at the full concentration ( $\sim 8 \times 10^4$  particles  $\text{cm}^{-3}$ ) of aerosol. The relative difference between the concentrations for each particle size bin is presented in Supplementary Figure 2. The minimum collection efficiency is  $> 98\%$  and the total collection efficiency of the filter, for the particle size distributions in Figure 3 at 30 cm distance, is  $> 99.8\%$ .



**Supplementary Figure 4. Particle collection efficiency of the filter material for particles between 0.2 and 5.0 μm.** Collection efficiency was 100% for all bins greater than 5.0 μm (measured up to 10.0 μm) but results were omitted from the graph.

## **Propagation and inactivation of coronaviruses**

### **Supplementary Method 1. In vitro virus propagation and inactivation.**

The SARS-CoV-2 and HCoV-NL63 viruses were cultured using monkey kidney cells (LLC-MK2) using Dulbecco's modified Eagle's Minimum Essential Medium (DMEM) 2% fetal bovine serum with gentamycin and mycostatin as culture medium. At time of harvesting 75% of cells were infected with the virus. After harvesting the virus was inactivated by heating for 2 hours at 60°C. To ensure the complete inactivation, the heat-treated viruses were re-cultured in LLC-MK2 cells. The cultured cell lines showed no cytopathogenic effects and the process was repeated in three independent batches to certify the inactivation of SARS-CoV-2.

## **Patient demographic data and test results**

**Supplementary Data. Patient data (.xlsx) and results for, "The SARS-CoV-2 viral load in COVID-19 patients is lower on face mask filters than on nasopharyngeal swabs."**

NA, not applicable. nd, not done.