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A Versatile Filter Test System to Assess Removal Efficiency for Viruses in Aerosols

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ABSTRACT

Mitigation measures to reduce indoor transmission of SARS-CoV-2 and other pathogenic microorganisms are urgently needed to combat the current pandemic and to prevent future airborne epidemics or pandemics. Very efficient exhaust filters for nanoparticles down to sizes of only a few nanometers have been available for many years; they are used, for example, in diesel and, more recently, gasoline vehicles to reduce emissions. The size of soot particles emitted by combustion engines, i.e., primary particles and aggregates, includes those of viruses. Therefore, such particle filters should also efficiently remove viruses. This study aimed to design a filter test system with a controlled airflow allowing to aerosolize particles at the aerosol inlet and collect samples before and after the particle filter.

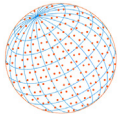
As an example, results obtained for the NanoCleaner[®], a filter designed to clean cabin air in vehicles, are presented. Validation with soot particles produced with a CAST soot generator revealed a filter efficiency higher than 99.5%. To assess the relevance of the test filter system to measure efficiency for viral particles removal, MS2 bacteriophages, also called *Escherichia* virus MS2, were used as virus surrogate and aerosolized into the filter test system with the commercially available Emser nebulizer. Filter efficiencies of more than 99% for MS2 bacteriophages were achieved using the NanoCleaner[®] in the filter test system. Experiments with ceramic wall-flow filters showed similar results. To enlighten the versatility of the filter test system, a typical aircraft cabin air filter was also characterized. The measurements confirmed the high filter efficiency, and in addition, we show a decrease of bacteriophage's survival on the filter material over 48 h post-exposure.

In conclusion, we have established a versatile system that is modular to test any filter system for the efficiency of eliminating MS2 bacteriophages as virus surrogates from air.

Keywords: Particle filter, MS2 bacteriophages, Virus aerosols, Filter efficiency, Filter test system

1 INTRODUCTION

In the current coronavirus pandemic, several air-borne routes of the SARS-CoV-2 virus transmission have been described such as (i) large droplets and micro-droplets in the range of 1000 nm expelled during coughing, sneezing and speaking, and (ii) exhaled aerosols containing single viruses in the 100 nm range released from breathing and speaking (Johnson and Morawska, 2009; Chu *et al.*, 2020; Riediker, 2020). Regarding aerosol transmission, the risk of SARS-CoV-2 outdoor transmission is much lower in comparison to indoor transmission (Bulfone *et al.*, 2020). Most transmissions occur in indoor environments (Nishiura *et al.*, 2020) and a recent systematic review has shown evidence that the virus is transmitted indoor via air, especially in hospitals and crowded places (Noorimotlagh *et al.*, 2020). Monte Carlo simulation scenarios revealed the significance of viral



load, speaking and physical activity to estimate the viral exposure and inhaled dose in different indoor scenarios (Riediker and Monn, 2020). Importantly, there is evidence that viruses are very efficiently spread through aerosols by the patient's breathing only (Scheuch, 2020) and even asymptomatic patients infected by SARS CoV-2 release a cloud of such viruses when breathing normally (Furukawa *et al.*, 2020). It has been shown that humans produce infectious aerosols in a wide range of particle sizes, but pathogens predominate in small particles below 5 μm (Fennelly, 2020). Depending on the conditions, the exhaled carrier droplets can dry in a fraction of a second and the smaller remaining particles then spread unhindered in the indoor air as an aerosol (Klompas *et al.*, 2020). However, exhaled aerosols also contain surfactants, lipids and proteins that can prevent this effect, and this has been shown to be largely dependent on relative humidity (Vejerano and Marr, 2018). Large droplets settle faster than they evaporate and can contaminate the immediate vicinity of the infected individual, whereas small droplets evaporate faster than they settle and can remain airborne for a much longer time (Bourouiba, 2020).

From the viewpoint of aerosol physics, free viruses or what remains after the evaporation process (de Oliveira *et al.*, 2021) are "biological" ultrafine particles. Like other such particles, they remain suspended in air for hours and can thus reach everyone in the same room. Therefore, keeping a distance in closed rooms is insufficient to prevent from infection once the virus has spread. Different mitigation measures have been proposed such as active ventilation with outside air, the ubiquitous wearing of face masks, and air ventilation (Nielsen, 2009; Lelieveld *et al.*, 2020). The latter can be done by supplying fresh air from outside or by filtering and recirculating the room air (Morawska *et al.*, 2020). The first possibility is the simplest but from an energetic point of view undesirable during cold or hot weather conditions. The second option requires adequate filters. In both cases it is essential that air is withdrawn from the room in a way avoiding the distribution of the viruses in the room. This can be achieved by a vertical flow such that exhaled air immediately moves upwards. Several filter systems have been reported recently with the aim to filter airborne viral particles. For instance, cellulose-based virus-retentive filters have been described (Junter and Lebrun, 2017) or the design of high-performance charged nanofiber filters that achieve over 90% capture of airborne SARS-CoV-2 simulated by the 100 nm sodium chloride aerosols (Leung and Sun, 2020).

In addition, soot particle filters have gained the interest in this field. Electron microscopy analysis of the corona virus SARS-CoV-2 revealed a spherical shape and size between 60 nm and 140 nm in diameter (Zhu *et al.*, 2020). This is in the same range as soot particles (Maricq *et al.*, 2000). The mean size for combustion-derived primary particles is in the order of 20 nm. These primary particles are emitted as agglomerates having a lognormal size distribution with a median of around 80 nm for diesel and 60 nm for gasoline engines (Mayer *et al.*, 2014). Therefore, particle filters such as those developed since the 1990's for the elimination of soot particles from the exhaust gas of diesel engines, and which have now become the state of the art in all vehicles with combustion engines, could also be suitable to filter viral particles (Mayer *et al.*, 1999). Such particle filters achieve separation efficiencies of well over 99%, are space-saving and cost-effective. An additional advantage of using such filters is that not only viruses, but also all particulate air pollutants such as soot particles are removed.

To apply such particle filters for the envisaged purpose, the efficiency of these filters to precipitate viruses needs to be investigated. Therefore, we developed a test bench with a controlled airflow allowing to aerosolize particles at the aerosol inlet and to collect samples before and after the filter. The first filter tested and reported here was a fiber-based nanofilter, the Nanocleaner[®], used so far for vehicle cabin air cleaning (Burtscher *et al.*, 2008). In a second step, a typical aircraft cabin air filter was used to assess the versatility of the system. The test system was validated with soot particles produced by a combustion soot generator (CAST). Then MS2 bacteriophages, also called *Escherichia coli* or Escherichia virus MS2, were used as virus surrogate to assess the filter efficiency for viral particles (virions) removal (Aranha-Creado and Brandwein, 1999). As mentioned SARS-CoV-2 viruses have a spherical shape and size between 60 nm and 140 nm in diameter (Zhu *et al.*, 2020). In comparison, the size of bacteriophage MS2 is around 30 nm (Nguyen *et al.*, 2011). Additionally, bacteriophages MS2 proved to be a good model system for corona virus studies as both are negatively charged (Diaz, 2008) and hence expected to have similar behaviour when interacting with the filter surface (Dang and Tarabara, 2019). Finally, MS2 bacteriophages are persistent in the environment and can be detected and counted accurately as active bacteriophages.

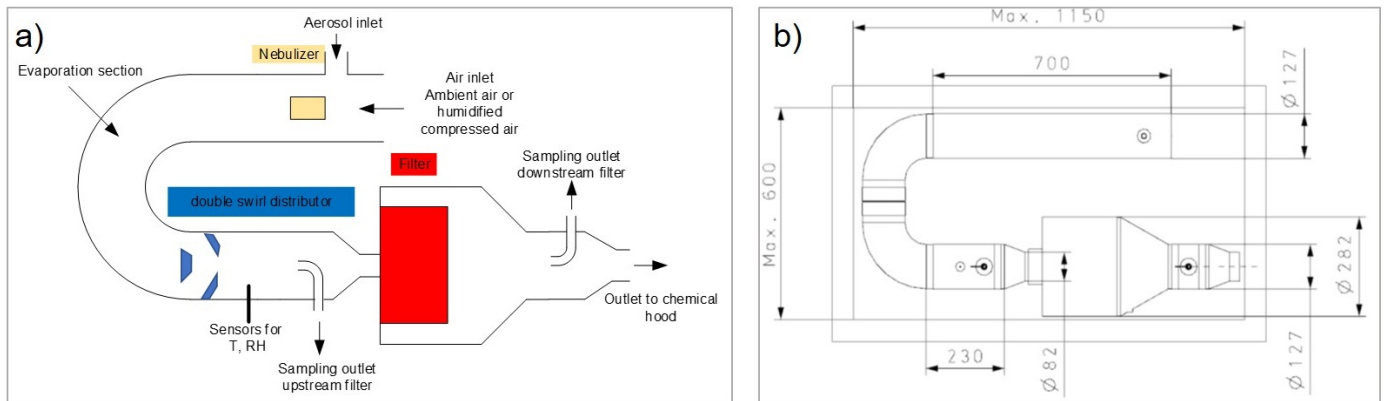
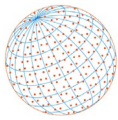


Fig. 1. Diagram of the test system. (a): configuration, not to scale, T: Temperature, RH: Relative humidity. The position of the probe upstream the filter can be varied, allowing the scan the whole cross section. (b): drawing with dimensions.

2 MATERIALS AND EXPERIMENTAL SETUP

2.1 Design of the Filter Test Bench

The filter test bench was designed to measure aerosol particles as well as MS2 bacteriophages as viral surrogates (Fig. 1). The dimensions were chosen such to allow flow rates up to $100 \text{ m}^3 \text{ h}^{-1}$. This covers a wide range of filters and the dimensions of the entire test stand are small enough to fit into a conventional chemical hood. The NanoCleaner[®] particle filter (Burtscher *et al.*, 2008), used here as an example requires a flow rate of about $20 \text{ m}^3 \text{ h}^{-1}$.

Aerosol particles were introduced into the main flow (ambient air or filtered compressed air) externally via the aerosol inlet. Particles and MS2 bacteriophages were introduced via a nebulizer. A medical inhalator (EMSER[®] Inhalator compact, <https://www.emser.ch/bronchien-lunge/produkte/emser-inhalator-compact/>) has been used as nebulizer to produce defined aerosols. It is operated at ambient pressure. The aerosol introduction is followed by an evaporation section. According to Drewnick and Team the evaporation time of a $10 \mu\text{m}$ droplet is about 0.2 s at 70% relative humidity (RH) (https://www.mpic.de/4670174/filtermasken_zusammenfassung.pdf). The residence time of the aerosol in the system (0.4 s at the maximum flow rate of $100 \text{ m}^3 \text{ h}^{-1}$) therefore is long enough for the droplets produced by the nebulizer to evaporate before reaching the measuring point at the filter inlet. A double swirl distributor insured a homogeneous distribution over the whole cross section.

Two sampling outlets upstream and downstream the filter allow measuring the particle size distributions and collecting the MS2 bacteriophages before and after the filter. The cross section of the suction pipes was chosen so that at $20 \text{ m}^3 \text{ h}^{-1}$ main flow and 5 L min^{-1} sample flow the sampling is isokinetic. The position of the sampling location upstream the filter is adjustable so that the homogeneity of the aerosol concentration can be measured. This is important to make sure that the sampling is representative. The whole system has to be cleaned with 70% alcohol and the next experiment can only be performed after 12 h post-exposure. Fig. 2 shows a picture of the test system, installed in the chemical hood.

In the experiment presented here the flow was controlled by the blower in the filter (here the NanoCleaner[®], originally developed for cleaning the cabin air in cars). To test other filters, external blower is used.

2.2 Tests with Aerosol Particles

In a first series of experiments, the filter efficiency was determined using soot particles, produced by a flame soot generator (CAST, Jing Ltd., sootgenerator.com, see also Ess and Vasilatou (2019)). To cover the size range of interest several settings of the CAST “producing different size distributions” were used. The size distribution was measured upstream and downstream the filter using a Scanning Mobility Particles Sizer (SMPS, DMA TSI 3081, CPC, TSI 3775). The main flow was $20 \text{ m}^3 \text{ h}^{-1}$, aerosol inlet flow 1.5 L min^{-1} , sample flow 5 L min^{-1} .

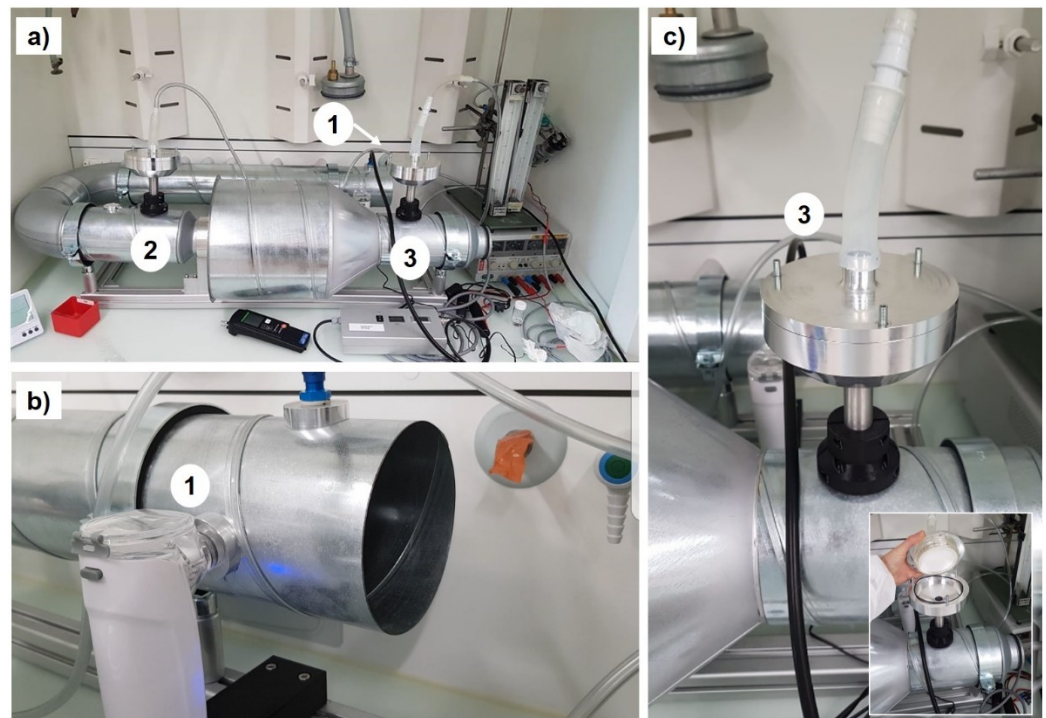
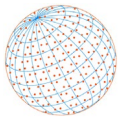


Fig. 2. NanoCleaner[®] particle filter test bench system. (a) Test bench system overview with the aerosolization point (1), the sampling outlet for the upstream filter (2) and the sampling outlet for the downstream filter; (b) Aerosolization point with the commercial Emser nebuliser (1); (c) Collection point at downstream filter outlet (3) and gelatine membranes (inset).

The filter is tested with face velocities as suggested by the manufacturer (about 1 cm s^{-1}).

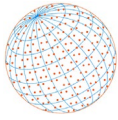
To make sure that aerosolized particles are completely dry after the evaporation section we produced NaCl particles by atomizing a NaCl solution and measured their size distribution at the sampling port upstream the filter directly and after passing them through a silica-gel dryer. The size distribution remained unchanged, showing that the particles already have been dry before entering the dryer. Scanning the particle concentration in the whole cross section by moving the location of sampling probe 1 proved that the concentration is homogeneous, independent of the exact position of the probe sampling will be representative.

2.3 Growth of *E. coli* Host Cultures and MS2 Bacteriophages

The *Escherichia coli* bacteriophage MS2 (ATCC[®] 15597B1[™]) and the *Escherichia coli* (*E. coli*) (ATCC[®] 15597[™]) host cultures were purchased from ATCC and cultured in 271 media according to the supplier instructions. Briefly, one day prior to the experiment, $10 \mu\text{L}$ of *E. coli* culture was added to 10 mL of 271 media and incubated at 37°C overnight at 160 rpm in a shaking incubator. The following morning 0.5 mL of overnight culture was added to 10 mL of fresh 271 media and further incubated until the exponential phase of bacterial growth was reached. The exponential phase was determined spectrophotometrically by an optical density (OD) 600 of 0.5 – 0.7 . The culture was then kept at room temperature until use.

2.4 Aerosolization of MS2 Bacteriophages and Sampling on Filters

The MS2 bacteriophages were kept at 4°C until use. On the day of the experiment, $100 \mu\text{L}$ of the original stock solution was added to 5 mL of warm 271 medium. Once prepared, this stock solution was aerosolized in the test system for 15 min using the commercially available Emser nebulizer. The time needed for all liquid to be nebulized was 12 min to ensure that all aerosolized bacteriophages reach the sample collection 1 (sampling port upstream the particle filter) and sample collection 2 (sampling port downstream filter) (Figs. 1 and 2). The average temperature during aerosolization was 21°C and humidity 40 – 50% .



During the aerosolization, the MS2 bacteriophage samples were collected on the gelatin membranes positioned at the two sample collection places. The gelatin membranes were then dissolved in 5 mL of 271 media, and 10-fold serial dilutions of MS bacteriophage were prepared (10^{-1} – 10^{-6}) by transferring 20 μL of bacteriophage sample to 180 μL of 271 media, and the viral titer was determined as described (Miller, 1972). Briefly, 100 μL of *E. coli* bacterial culture was pipetted in individual tubes and the 100 μL of bacteriophage sample were added. The aliquots were incubated for 10 min at 37°C before 5 mL of the soft top agar was added, and transferred onto the pre-heated solid agar plates and incubated overnight. On the following day, the plaques were counted and the viral titer determined as plaque forming units (PFU) by serial dilutions.

To calculate the filter efficiency (%) the following formula was used:

$$\text{Filter efficiency (\%)} = 100 - \frac{\text{PFU/mL sampled after the NanoCleanAir}^{\text{®}} \text{ particle filter} \times 100}{\text{PFU/mL sampled before the NanoCleanAir}^{\text{®}} \text{ particle filter}} \quad (1)$$

2.5 Control Experiments

A plate with *E. coli* only was included to eliminate the possibility of viral contamination during the plate preparation. Furthermore, the experiments described for the optimized methods on gelatin membranes were kept on the bench in the hood throughout the experiment and it served as the environmental control to assess potential MS2 bacteriophage contamination in the air. The samples from the MS2 bacteriophage stock solution were also prepared to serve as a positive control.

2.6 Transmission Electron Microscopy (TEM) of Bacteriophages

The MS2 bacteriophage stock solution prepared as described in section 2.4 was used for the microscopy experiments. As described elsewhere (Ackermann and Tiekotter, 2012), a drop of 5 μL was pipetted onto 200 mesh TEM copper grids coated with a carbon/formvar film (Emsdium, Pennsylvania, USA) for the negative staining. The grids were left 3 min at room temperature to let the bacteriophages absorb to the surface. Then, the grids are manually blotted with a Whatman[®] filter paper (Sigma-Aldrich, Switzerland), and placed into a 5 μL drop of 2% phosphotungstic acid pH 7.0 for 45 s. By using wet Whatman[®] filter paper, the excess of staining agent is removed to leave a thin layer of stain onto the bacteriophages. The samples were air dried 12 h before imaging. The grids were examined with a Tecnai spirit TEM (ThermoFischer, Oregon, USA) operating at 120 kV, with a FEI eagle camera (ThermoFisher, USA) using the TIA software (ThermoFisher, Oregon, USA).

3 RESULTS

3.1 Test Measurements with Soot Particle Aerosols

A first series of measurements have been done with aerosol soot particles. Particles were generated with a CAST soot generator (CAST: Combustion Aerosol Standard, www.sootgenerator.com), filtered compressed air was introduced. A Scanning Mobility Particle Sizer (SMPS) was used to alternately measure the particle size distribution before and after the filter. Particle production by the CAST is stable, no significant changes were observed during the experiment. Fig. 3(a) shows the measured size distributions upstream and downstream the filter for two settings of the CAST, and Fig. 3(b) the filtration efficiency. The achieved average filter efficiency is higher than 99.5%. Varying the sampling location shows that the distribution over the complete cross sections is absolutely homogeneous.

3.2 Determination of Filter Efficiency Using MS2 Bacteriophages

The MS2 bacteriophage solution was aerosolized in the test system for 15 min using the commercially available Emser nebulizer under the defined flow and environmental air stream. After the aerosolization, the MS2 bacteriophage samples were collected on gelatin membranes. The membranes were then dissolved and the PFUs were determined as described in the experimental section. The aerosolization experiments were repeated five times and the results are summarized

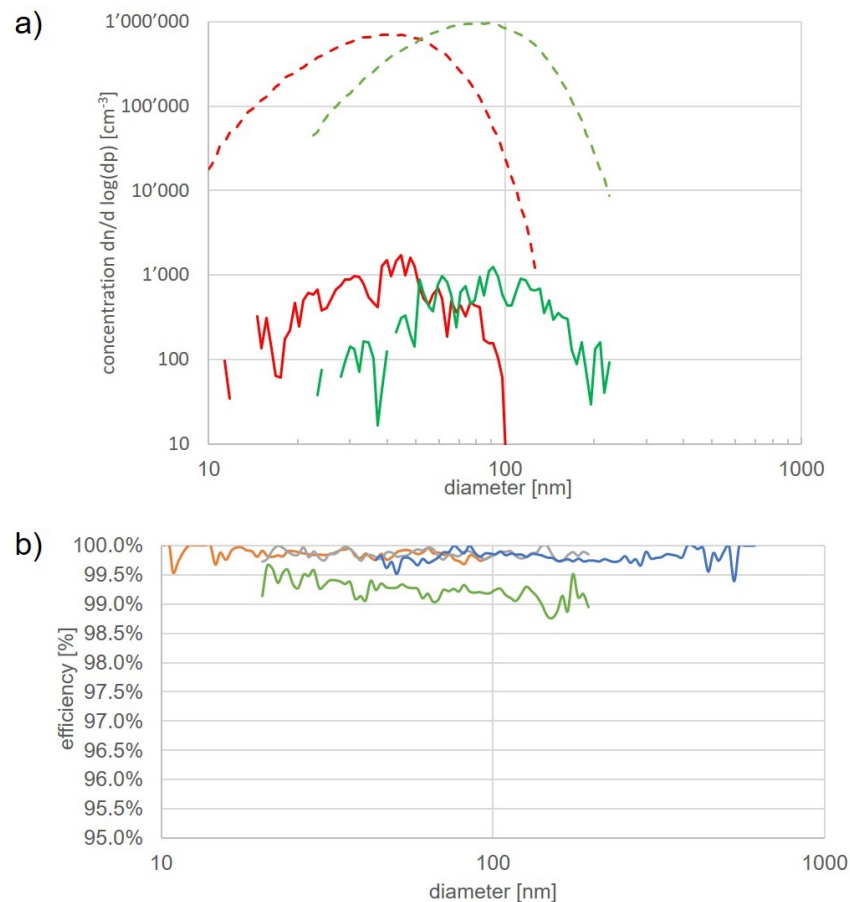
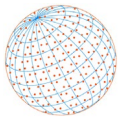


Fig. 3. (a) Size distributions of aerosols with and without filter. Dotted lines upstream of the filter, solid lines downstream the filter (flow $18 \text{ m}^3 \text{ h}^{-1}$, face velocity 1 cm s^{-1}); red: CAST setting 27 nm, green: 88 nm average diameter. (b) Filter efficiency measured at a flow rate of 18 L min^{-1} (0.6 L s^{-1}) at three different CAST settings (orange 27 nm, gray 88 nm, blue 105 nm average diameter) to best represent the particle diameter of interest. The small differences show that the measurement is very robust. The green curve shows a measurement at $28 \text{ m}^3 \text{ h}^{-1}$ (1.55 cm s^{-1}). The efficiency is slightly lower, but still very high.

in Figs. 4 and 5. The results show that even at the lowest dilution PFUs representing active bacteriophage particles are absent in the sample collected after NanoCleaner[®] filter (sampling downstream filter) (Fig. 4). From the number of plaques, it can be observed that the filter efficiency of bacteriophage elimination is $> 99\%$ (Fig. 5).

3.3 Control Experiments

The hood was always cleaned with 70% ethanol at the end of each experiment to avoid any MS2 bacteriophage contamination in the bench, which could result in false plaque formation. Due to potentially easy contamination of the surrounding air with the MS2 bacteriophages, apart from the standard negative control used to assess MS2 bacteriophage contamination in bacterial culture, an additional control to check for the contamination in surrounding air has been performed. An opened gelatine filter was placed on the bench during aerosolization and served as an environmental control (Fig. 6) to monitor the contamination in the air. No plaques were observed for this control.

3.4 Versatility of the Bench Test Filter System

The test bench system is a modular system and the versatility of the set-up was exploited to characterise a typical aircraft cabin air filter to filter viral particles (Fig. 7(a)). The measurements were done similarly as indicated for the NanoCleaner[®] particle filter. Over the two repetitions, we

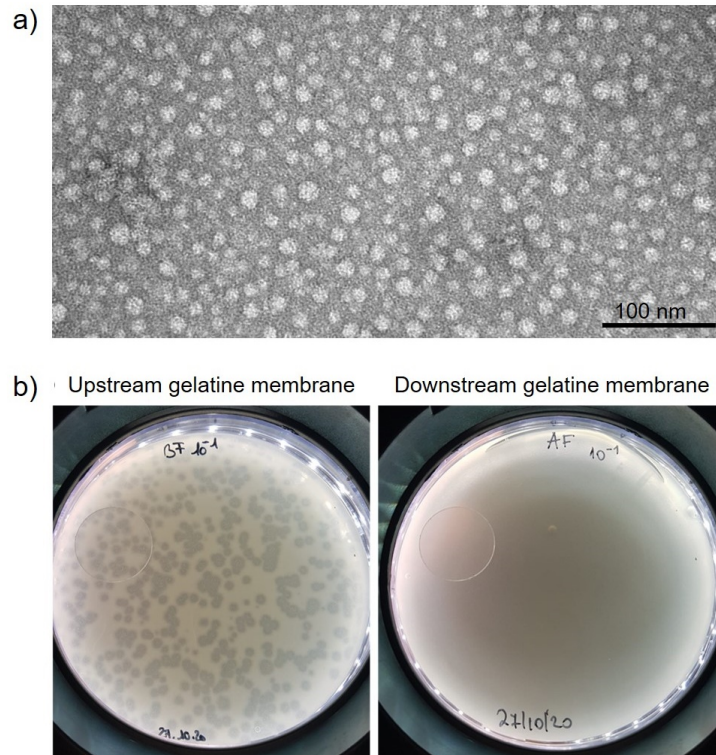
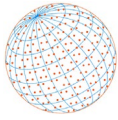


Fig. 4. (a) Transmission electron micrograph of bacteriophages MS2 from the initial stock solution. (b) Representative photographic images of the agar plates from which the number of plaques was counted from MS2 bacteriophages collected on the upstream and downstream gelatine membranes.

could not detect any PFUs after the filter (Fig. 7(b)). These measurements confirmed a filtering efficiency > 99% for MS2 bacteriophages in the tested conditions.

Another advantage of this filter is that the evaluated filtering system can be retrieved after the aerosolization, and therefore, the MS2 bacteriophages survival on the filter can be assessed. As presented in Fig. 7(c), the survival of the MS2 bacteriophage on the filter, 24 and 48 h after aerosolization, varies between the two experimental replicates. Nevertheless, a steady decrease is observed over the 48 h post aerosolization. This information is essential to ensure that the filtering device is not becoming a source of contamination over time.

4 DISCUSSION

Indoor airborne transmission of SARS-CoV-2 should be minimized by reducing the concentration of the virus in the air, especially in crowded rooms (Noorimotlagh *et al.*, 2020). The option remains to continuously filter the room air in circulation in order to continuously remove all nanoparticles including viral particles from the air. Particulate filters in indoor environments have been described but they are intended to remove larger particles (Morawska *et al.*, 2020). Most air purifiers employ high-efficiency particulate air filters (HEPA) and it has been shown that the filtration efficiencies of HEPA is high enough to remove virus-laden aerosols released by COVID-19 patients via sneezing, coughing or breathing (Chen *et al.*, 2019; Zhao *et al.*, 2020).

Nanofilters introduced in exhaust engines could also be suitable for this purpose, as they have been developed since the 1990's for the elimination of soot particles from the exhaust gas of diesel and gasoline engines and have now become state of the art for all vehicles with combustion engines (Miller and Jin, 2018). They achieve separation efficiencies of well over 99%, have a space-saving design (> 1 m² of filter area per liter of construction volume), and are cost-effective (Majewski and Khair, 2006). So far, the application of these filters has been limited to exhaust gas from internal combustion engines. However, it is to be expected that viruses, which are in

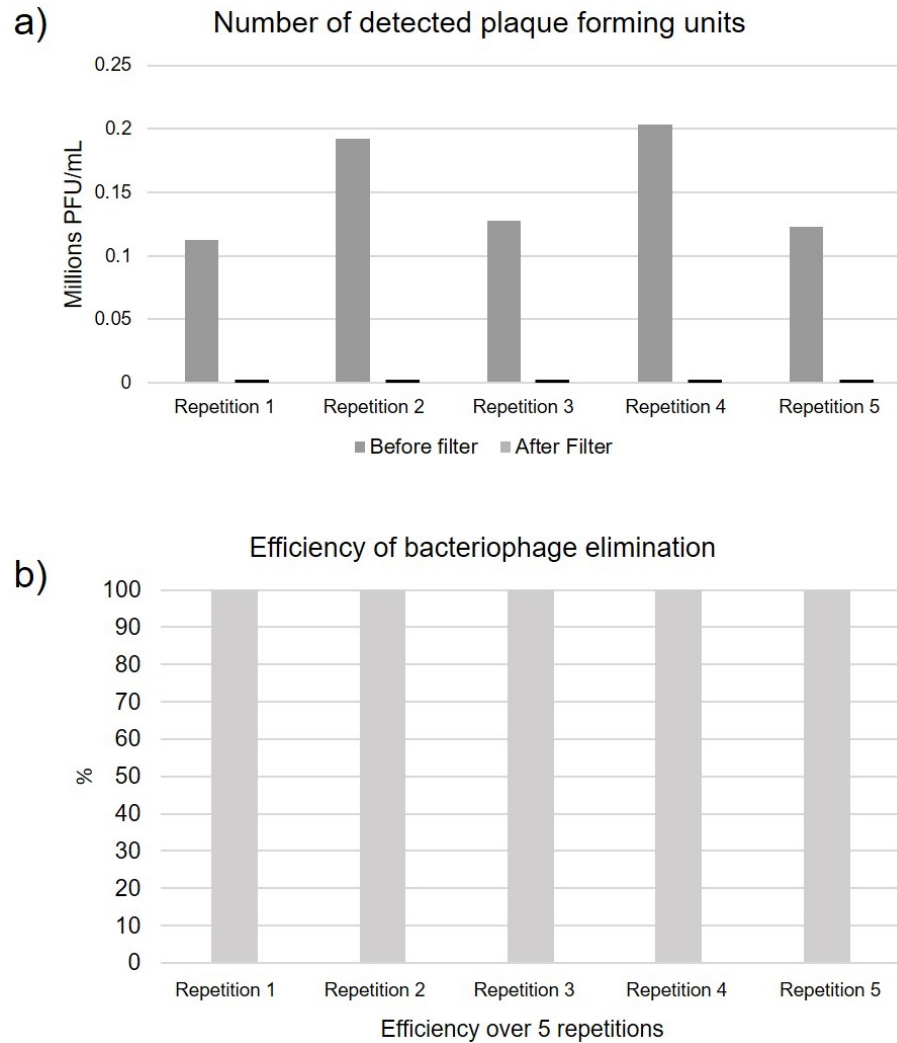
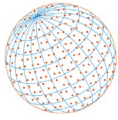


Fig. 5. (a) Number of detected plaque forming units before the NanoCleaner® filter (upstream filter, grey bars) and after (downstream filter, black bars). No plaques were detected on the downstream gelatine filter. (b) Efficiency of bacteriophage elimination over 5 repetitions.

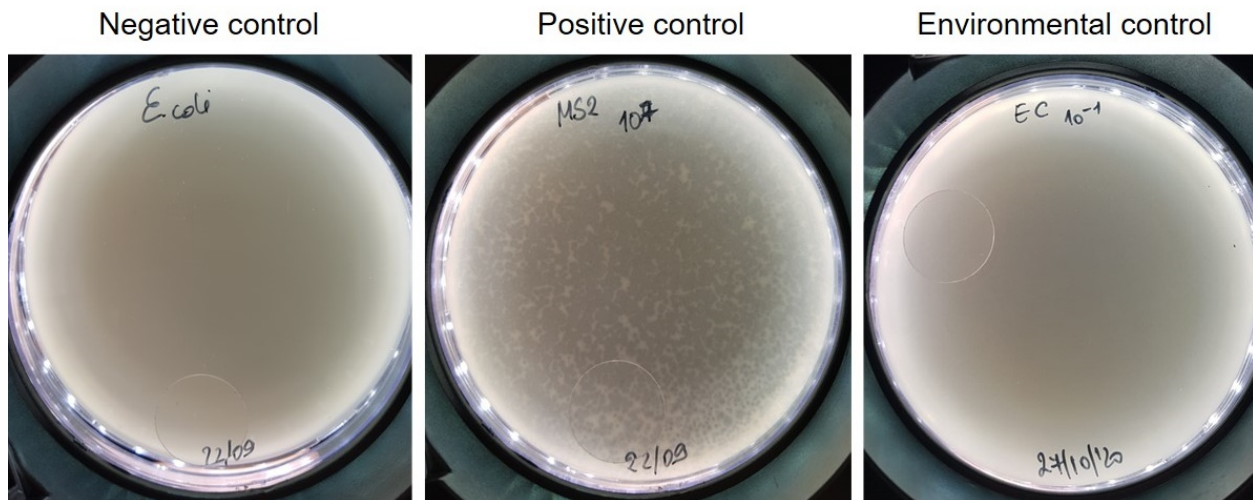


Fig. 6. Representative photographic images of the agar plates which served as the control: negative control (bacterial host only), MS2 (bacteriophage stock solution incubated with the host) and environmental control (EC) which served to monitor the air during the experiment execution.

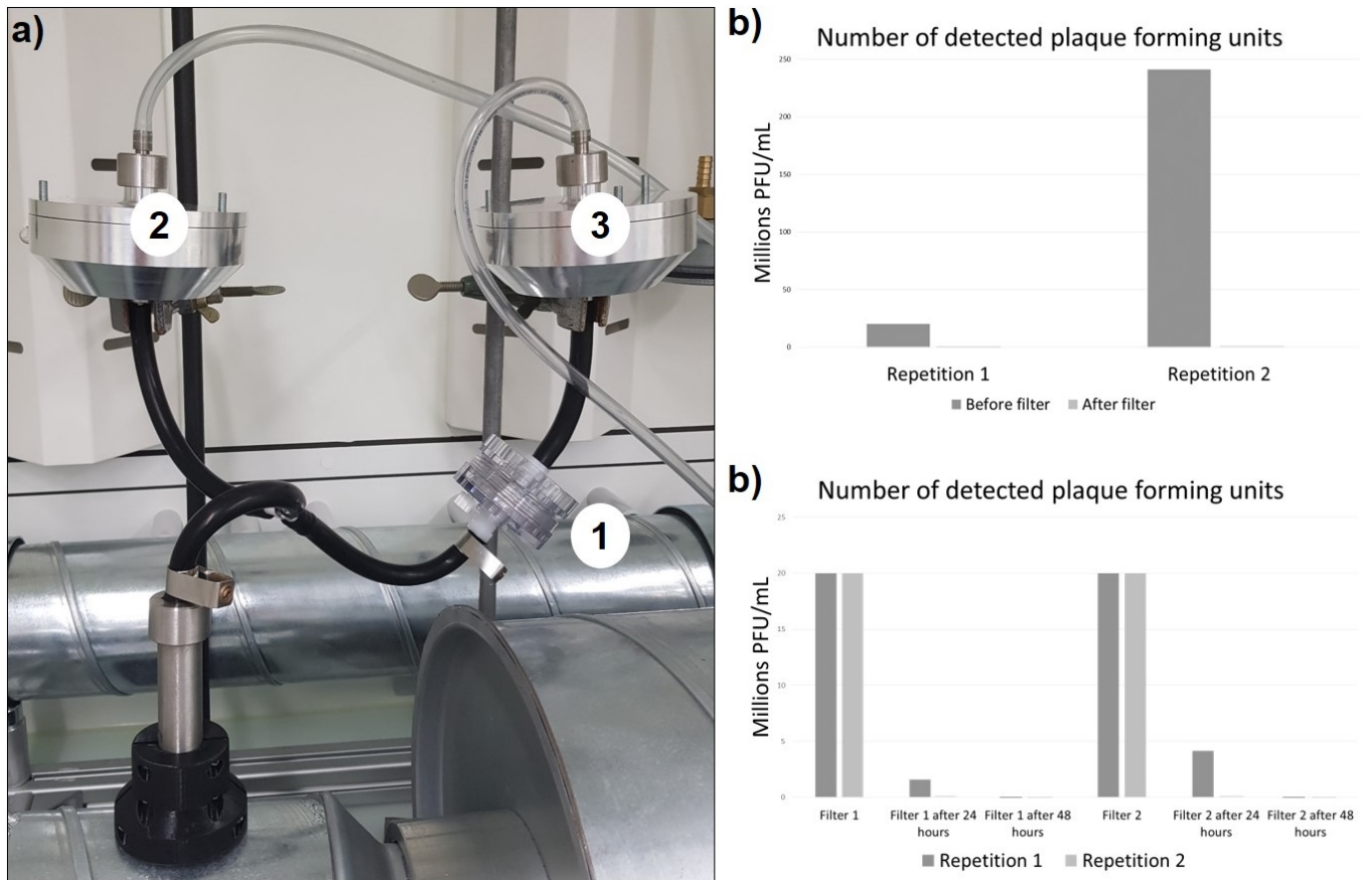
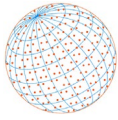
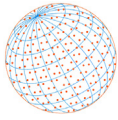


Fig. 7. (a) Test bench system modification to test a typical aircraft cabin air filter cast inside a plastic filter holder (1). The sampling outlet for the upstream filter (2) and downstream filter outlet (3) are collected on gelatine filters. (b) Number of detected PFUs before the aircraft cabin air filter (upstream filter, dark grey bars) and after (downstream filter, light grey bars). (c) Survival of the MS2 bacteriophages on the aircraft cabin air filter after 24 and 48 h post-exposure.

the size range of single and agglomerated soot particles, will behave analogously to these and can therefore be eliminated by similar means.

The test measurements showed that our designed filter test system meets the requirements with regard to aerosol measurements. The particle concentration is homogeneous in the entire cross-section. The residence time is long enough so that NaCl particles, produced by nebulization of a NaCl-solution, arrive dry at the measuring point at the filter inlet. In addition, the setup is modular, so it can be adapted to other filter geometry without much effort. As expected, the filter efficiency for soot particles generated with the CAST generator was very high, at $18 \text{ m}^3 \text{ h}^{-1}$ over the entire measured size range greater than 99.5%. Since the minimum of the filter efficiency was in the measured range, the efficiency will be even higher for smaller and larger particles.

The work with pathogenic viruses and especially with highly transmissible viruses poses very big technical challenges. Due to the high contagiousness, transmissibility, and virulence of SARS viruses in general and SARS-CoV-2 in particular, experiments would have to be carried out under the highest biosafety measures (BSL-3/4), which makes aerosol experiments practically impossible. In addition, the detection of still active viruses is very difficult and also not very meaningful, because the live detection methods for viruses have a low sensitivity. In addition, very complex cultivation methods with special human epithelial cells are necessary for human pathogenic viruses. For these reasons, many researchers in environmental biology use bacteriophages as surrogate viruses (proxy viruses) (Aranha-Creado and Brandwein, 1999). Bacteriophages are morphologically, structurally, and genetically similar to human viruses such as SARS-CoV-2, noroviruses or rotaviruses, so that bacteriophages can be used as safe surrogates for human viruses. To assess the filter efficiency for viral particles (virions) removal, the bacteriophage MS2, also called Escherichia virus MS2, was used. MS2 bacteriophage infects and replicates only in *Escherichia*



coli F⁺ K12 bacteria, a subclass of *Escherichia coli* that is a safety class 1 laboratory stain, making it hence a bacteriophage, which is safe for human handling. Furthermore, MS2 bacteriophages are persistent in the environment including the air and are significantly smaller than other known human viruses and hence are particularly interesting to assess filtration cut-off for various filter purification systems. Electron microscopy of the corona virus SARS-CoV-2 revealed their spherical shape and size between 60 nm and 140 nm in diameter (Zhu *et al.*, 2020). On the other hand, the size of MS2 bacteriophage is around 30 nm. Additionally, MS2 bacteriophages proved to be a good model system for virus studies as both are negatively charged and hence expected to have similar behavior when interacting with the filter surface (Diaz, 2008).

We have shown that the test bench system can also be used to aerosolize the virus model particle, i.e., MS2 bacteriophages, into the air stream. The collection of the bacteriophages MS2 on gelatine membranes, which are sampled before and after the filter (i.e., gelatine membrane 1 and gelatine membrane 2), allows to determine the filter efficiency. The negative and environmental controls we introduced revealed that the system can be efficiently cleaned by aerosolization of 70% ethanol after MS2 bacteriophage aerosolization and that the system is completely tight as no plaque formation was observed when surrounding air was tested. We also introduced an additional cleaning of the hood with 70% ethanol after each experiment including a several hour waiting time before the next experiment was performed. Finally, the optimisation of the MS2 bacteriophage aerosolization, the cleaning procedure after each experiment and the determination of the PFUs resulted in highly reproducible results. With the optimised approach, we could demonstrate a NanoCleaner[®] filtering efficiency for MS2 bacteriophages of > 99%. Here we only presented results from fiber filters; however, experiments with ceramic wall-flow filters as used for diesel particle filters show the same results.

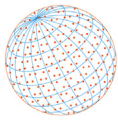
As a next step we assessed the versatility of the system by introducing a typical aircraft cabin air filter. Again, a filter efficiency of > 99% for MS2 bacteriophages for this kind of filter could be shown. This filter also had the advantage that we could explore the MS2 bacteriophage survival after the aerosolization process. The filtering system could be retrieved after the aerosolization by cutting the filter into several pieces, which was not possible with the NanoCleaner[®] system. We demonstrated a significant lower PFU over the 48 h post aerosolization indicating that the activity of MS2 bacteriophages is decreasing within a short period of time.

5 CONCLUSIONS

This project aimed to verify the particle filter efficiency of airborne MS2 bacteriophages using a novel test bench with an aerosol inlet and two sampling outlets to sample MS2 bacteriophages before and after a filter. The measurement campaign with combustion soot aerosols showed separation efficiencies > 99.5% in the entire alveolar relevant size range 10–300 nm, which can be improved in further filter systems. In addition, we have succeeded in releasing MS2 bacteriophages as surrogate viruses for SARS CoV-2 viruses in an air stream and implemented the methodology of sampling and numerical determination of the virus activity. Filter efficiencies of more than 99% for MS2 bacteriophages were achieved using the NanoCleaner[®] particle filter. The versatility of the system was demonstrated with an aircraft cabin air filter confirming the high filter efficiency in this system, and in addition the MS2 bacteriophage activity was significantly decreasing over 48 h post aerosolization time. In conclusion, we have shown that the NanoCleaner[®] particle and the typical aircraft cabin air filter system could be used in indoor surroundings as a potential air purification system to reduce airborne viral transmission.

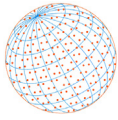
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REFERENCES

- Ackermann, H.W., Tiekotter, K.L. (2012). Murphy's law-if anything can go wrong, it will: Problems in phage electron microscopy. *Bacteriophage* 2, 122–129. <https://doi.org/10.4161/bact.20693>
- Aranha-Creado, H., Brandwein, H. (1999). Application of bacteriophages as surrogates for mammalian viruses: A case for use in filter validation based on precedents and current practices in medical and environmental virology. *PDA J. Pharm. Sci. Technol.* 53, 75–82. <https://journal.pda.org/content/53/2/75.short>
- Bourouiba, L. (2020). Turbulent gas clouds and respiratory pathogen emissions: Potential implications for reducing transmission of COVID-19. *JAMA* 323, 1837–1838. <https://doi.org/10.1001/jama.2020.4756>
- Bulfone, T.C., Malekinejad, M., Rutherford, G.W., Razani, N. (2020). Outdoor transmission of SARS-CoV-2 and other respiratory viruses: A Systematic review. *J. Infect. Dis.* 223, 550–561. <https://doi.org/10.1093/infdis/jiaa742>
- Burtscher, H., Loretz, S., Keller, A., Mayer, A., Kasper, M., Artley, R.J., Strasser, R., Czerwinski, J. (2008). Nanoparticle filtration for vehicle cabins. SAE Paper 2008-01-0827. <https://doi.org/10.4271/2008-01-0827>
- Chen, C., Ji, W., Zhao, B. (2019). Size-dependent efficiencies of ultrafine particle removal of various filter media. *Build. Environ.* 160, 106171. <https://doi.org/10.1016/j.buildenv.2019.106171>
- Chu, D.K., Akl, E.A., Duda, S., Solo, K., Yaacoub, S., Schünemann, H.J., Chu, D.K., Akl, E.A., Elharakeh, A., Bognanni, A., Lotfi, T., Loeb, M., Hajizadeh, A., Bak, A., Izcovich, A., Cuello-Garcia, C.A., Chen, C., Harris, D.J., Borowiack, E., Chamseddine, F., *et al.* (2020). Physical distancing, face masks, and eye protection to prevent person-to-person transmission of SARS-CoV-2 and COVID-19: A systematic review and meta-analysis. *Lancet* 395, 1973–1987. [https://doi.org/10.1016/S0140-6736\(20\)31142-9](https://doi.org/10.1016/S0140-6736(20)31142-9)
- Dang, H.T.T., Tarabara, V.V. (2019). Virus deposition onto polyelectrolyte-coated surfaces: A study with bacteriophage MS2. *J. Colloid Interface Sci.* 540, 155–166. <https://doi.org/10.1016/j.jcis.2018.12.107>
- de Oliveira, P.M., Mesquita, L.C.C., Gkantonas, S., Giusti, A., Mastorakos, E. (2021). Evolution of spray and aerosol from respiratory releases: Theoretical estimates for insight on viral transmission. *Proc. R. Soc. A* 477, 20200584. <https://doi.org/10.1098/rspa.2020.0584>
- Diaz, M. (2008). Zeta Potential and Size Measuring of MS2 Virus Under Different pH and Ionic Strength. Proceedings of 2008 Annual Meeting for American Institute of Chemical Engineering, Philadelphia, Pennsylvania.
- Ess, M.N., Vasilatou, K. (2019). Characterization of a new miniCAST with diffusion flame and premixed flame options: Generation of particles with high EC content in the size range 30 nm to 200 nm. *Aerosol Sci. Technol.* 53, 29–44. <https://doi.org/10.1080/02786826.2018.1536818>
- Fennelly, K.P. (2020). Particle sizes of infectious aerosols: Implications for infection control. *Lancet Respir. Med.* 8, 914–924. [https://doi.org/10.1016/S2213-2600\(20\)30323-4](https://doi.org/10.1016/S2213-2600(20)30323-4)
- Furukawa, N.W., Brooks, J.T., Sobel, J. (2020). Evidence supporting transmission of severe acute respiratory syndrome coronavirus 2 while presymptomatic or asymptomatic. 26. *Emerging Infect. Dis.* <https://doi.org/10.3201/eid2607.201595>
- Johnson, G.R., Morawska, L. (2009). The mechanism of breath aerosol formation. *J. Aerosol Med. Pulm. Drug Delivery* 22, 229–237. <https://doi.org/10.1089/jamp.2008.0720>
- Junter, G.A., Lebrun, L. (2017). Cellulose-based virus-retentive filters: A review. *Rev. Environ. Sci. Biotechnol.* 16, 455–489. <https://doi.org/10.1007/s11157-017-9434-1>
- Klompas, M., Baker, M.A., Rhee, C. (2020). Airborne transmission of SARS-CoV-2: Theoretical considerations and available evidence. *JAMA* 324, 441–442. <https://doi.org/10.1001/jama.2020.12458>
- Lelieveld, J., Helleis, F., Borrmann, S., Cheng, Y., Drewnick, F., Haug, G., Klimach, T., Sciare, J., Su, H., Poschl, U. (2020). Model calculations of aerosol transmission and infection risk of COVID-19 in indoor environments. *Int. J. Environ. Res. Public Health* 17, 8114. <https://doi.org/10.3390/ijerph17218114>
- Leung, W.W.F., Sun, Q. (2020). Electrostatic charged nanofiber filter for filtering airborne novel



- coronavirus (COVID-19) and nano-aerosols. *Sep. Purif. Technol.* 250, 116886. <https://doi.org/10.1016/j.seppur.2020.116886>
- Majewski, W.A., Khair, M.K. (2006). Diesel emissions and their control. SAE International.
- Maricq, M.M., Podsiadlik, D.H., Chase, R.E. (2000). Size distributions of motor vehicle exhaust PM: A comparison between ELPI and SMPS measurements. *Aerosol Sci. Technol.* 33, 239–260. <https://doi.org/10.1080/027868200416231>
- Mayer, A., Kasper, M., Czerwinski, J. (2014). Nanoparticle counts emissions of trucks: EURO 3 with and without DPF compared to EURO 4 and EURO 5. *Energy Power* 4, 1–10. <https://doi.org/10.5923/s.ep.201401.01>
- Mayer, A., Matter, U., Scheidegger, G., Czerwinski, J., Wyser, M., Kieser, D., Weidhofer, J. (1999). Particulate traps for retro-fitting Construction site engines VERT: Final measurements and implementation. *SAE Trans.* 108, 407–420. <https://doi.org/10.4271/1999-01-0116>
- Miller, J. (1972). *Experiments in molecular genetics*. Cold Spring Harbor, N. Y.
- Miller, J., Jin, L. (2018). Global progress toward soot-free diesel vehicles in 2018. Global progress toward soot-free diesel vehicles. <https://doi.org/10.13140/RG.2.2.23212.62080>
- Morawska, L., Tang, J.W., Bahnfleth, W., Bluyssen, P.M., Boerstra, A., Buonanno, G., Cao, J., Dancer, S., Floto, A., Franchimon, F., Haworth, C., Hogeling, J., Isaxon, C., Jimenez, J.L., Kurnitski, J., Li, Y., Loomans, M., Marks, G., Marr, L.C., Mazzeo, L., *et al.* (2020). How can airborne transmission of COVID-19 indoors be minimised? *Environ. Int.* 142, 105832. <https://doi.org/10.1016/j.envint.2020.105832>
- Nguyen, T.H., Easter, N., Gutierrez, L., Huyett, L., Defnet, E., Mylon, S.E., Ferri, J.K., Viet, N.A. (2011). The RNA core weakly influences the interactions of the bacteriophage MS2 at key environmental interfaces. *Soft Matter* 7, 10449–10456. <https://doi.org/10.1039/C1SM06092A>
- Nielsen, P.V. (2009). Control of airborne infectious diseases in ventilated spaces. *J. R. Soc. Interface* 6, S747–S755. <https://doi.org/10.1098/rsif.2009.0228.focus>
- Nishiura, H., Oshitani, H., Kobayashi, T., Saito, T., Sunagawa, T., Matsui, T., Wakita, T., Suzuki, M. (2020). Closed environments facilitate secondary transmission of coronavirus disease 2019 (COVID-19). *medRxiv*, 2020.2002.2028.20029272. <https://doi.org/10.1101/2020.02.28.20029272>
- Noorimotlagh, Z., Jaafarzadeh, N., Martínez, S.S., Mirzaee, S.A. (2020). A systematic review of possible airborne transmission of the COVID-19 virus (SARS-CoV-2) in the indoor air environment. *Environ. Res.* 193, 110612–110612. <https://doi.org/10.1016/j.envres.2020.110612>
- Riediker, M. (2020). Nano-safety research lessons for dealing with aerosol transmissions of COVID-19. *Nanotoxicology* 14, 866–868. <https://doi.org/10.1080/17435390.2020.1786185>
- Riediker, M., Monn, C. (2020). Simulation of SARS-CoV-2 aerosol emissions in the infected population and resulting airborne exposures in different indoor scenarios. 21, 200531. <https://doi.org/10.4209/aaqr.2020.08.0531>
- Scheuch, G. (2020). Breathing is enough: For the epread of influenza virus and SARS-CoV-2 by breathing only. *J. Aerosol Med. Pulm. Drug Delivery* 33, 230–234. <https://doi.org/10.1089/jam.2020.1616>
- Zhao, B., Liu, Y., Chen, C. (2020). Air purifiers: A supplementary measure to remove airborne SARS-CoV-2. *Build. Environ.* 177, 106918–106918. <https://doi.org/10.1016/j.buildenv.2020.106918>
- Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., Niu, P., Zhan, F., Ma, X., Wang, D., Xu, W., Wu, G., Gao, G.F., Tan, W. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med.* 382, 727–733. <https://doi.org/10.1056/NEJMoa2001017>