CZECH TECHNICAL UNIVERSITY IN PRAGUE

DOCTORAL THESIS STATEMENT

Czech Technical University in Prague
Faculty of Biomedical Engineering
Department of Health Care Disciplines
and Population Protection

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PERFORMANCE OF DIFFERENT TYPES OF AIR FILTERS IN INFECTIOUS AGENTS INTERCEPTION

Ph.D. Programme: Population Protection
Branch of study: Civil Emergency Preparedness

Doctoral thesis statement for obtaining the academic title “Doctor of Philosophy”, abbreviated to “Ph.D.”

Kladno, March 2024

The work was produced in the part-time Ph.D. study at the Department of Biomedical Technology of the Faculty of Biomedical Engineering of the CTU in Prague.

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1. State of the Art

Currently, the possible ways of disease transmission and severe infectious diseases are studied profoundly to interrupt epidemics or highly contagious pathogen importation to distant destination. From history to current days the microorganisms were feared much and studied accordingly. In the ancient times the epidemics developed slower, but highly contagious pathogens could give rise to pandemics (plaque, variola). Only isolated adventurers could reach distant destinations and the chance to spread any exotic highly contagious pathogen was reduced by time consuming means of transport.

Nowadays nearly everybody can travel by airplane, nearly everybody can reach exotic destinations. The mobility of people is rising each day, the air travel spares time and shortens distances. Both advantages can convert in significant threats. Fast pathogen importation and fast infectious disease spread may occur. In addition, in developed countries with advanced medical care, many immunocompromised persons represent easy and highly sensitive targets for dangerous pathogens as well. The aircraft represents firstly the means of importation of pathogens. Secondly, the pathogens may spread from infected passenger to other persons onboard.

1.1. The Aircraft Air Conditioning System

The air conditioning system main function is to keep the air in the pressurized fuselage compartments at the correct pressure and temperature. Flying in a hostile environment, the air conditioning system is an essential device to keep sufficient oxygen level and comfortable temperature onboard. It consists of several key parts which provide tolerable conditions. In details, this system provides the following functions:

- cabin temperature control
- pressurization control
- avionics ventilation

1.2. Air Contaminants

1.2.1.1. Microbial Contamination Typical for the Aircraft Cabin

Majority of passenger aircraft fly at the height 8-12 km above the ground level. The air itself is a hostile environment for microorganisms, there is lack of humidity, energy sources and in high troposphere, where the aircraft operate, the ozone and temperature about -57°C (Klaban 2018) does not provide optimal conditions for living organisms, middle and upper troposphere can contain a wide

variety of microorganisms which can originate from oceans, soil or freshwater. The microbial species *Methylobacteriaceae* or *Oxalobacteraceae* found (DeLeon-Rodriguez et al. 2013) in high altitudes does not represent a threat for humans. The study of National Aeronautics and Space Administration (NASA) collecting the air samples from 0.3 to 12 km above the ground level revealed many bacterial genera present in high altitudes. *Mogibacterium*, *Bacteroides*, *Prevotella*, *Parabacteroides*, and *Corynebacterium* were detected. Additionally, the sporogenic bacteria as *Clostridium* and *Bacillus* were detected in abundance (Smith et al. 2018). But the bleed air generated by jet engines reaches temperatures between 200-300°C what makes the outside air going to the PACK and then to the cabin nearly sterile.

So, the main source of various microorganisms onboard is represented by humans. The bacteria comprised in aircraft microbiome usually are derived from human skin and oral commensals or the intestinal flora as well and can be found in the cabin air or on touch surfaces. The next bar chart shows the bacterial species contaminating both air and touch surfaces. The data obtained from long- haul flights simulating transcontinental flights show many bacterial species in the air or touch surfaces (Weiss et al. 2018). Among the genera comprising pathogens *Enterobacteriaceae*, *Pseudomonadaceae*, *Streptococccaceae* or *Staphylococcaceae* were detected especially in the cabin air. Table 1 summarizes Disease-associated bacteria detected in the cabin air.

Table 1 Disease-associated bacteria detected in the cabin air (Osman et al. 2008)

Acinetobacter calcoaceticus
Acinetobacter junii
Gemella haemolysans
Staphylococcus haemolyticus
Streptococcus mitis

1.2.2. Transmission of Airborne Pathogens in the Aircraft

Air travel serves as a conduit for infectious disease spread, including emerging infections. Current studies strongly suggest the insufficiency of existing air filters and the possibility of disease transmission from an infected passenger, so the threat of spread of infectious diseases in enclosed air-conditioned spaces should draw our vigil attention.

To review the air born infections possibly transmitted within the air-conditioned area of commercial aircraft, the Table 2 lists the number of cases and causative pathogen of inflight gained infection.

Table 2 Reported infections transmitted on commercial airlines. Adapted according to (Mangili and Gendreau 2005)

	Number of reports	Comments
Tuberculosis	2	Positive TB skin test only. No active TB.
SARS	4	No cases since WHO guidelines.
Common cold	0	Difficult to investigate.
Influenza	2	None since ventilation regulations.
Measles	3	Imported cases and international adoptions

1.3. Automotive Air Conditioning System

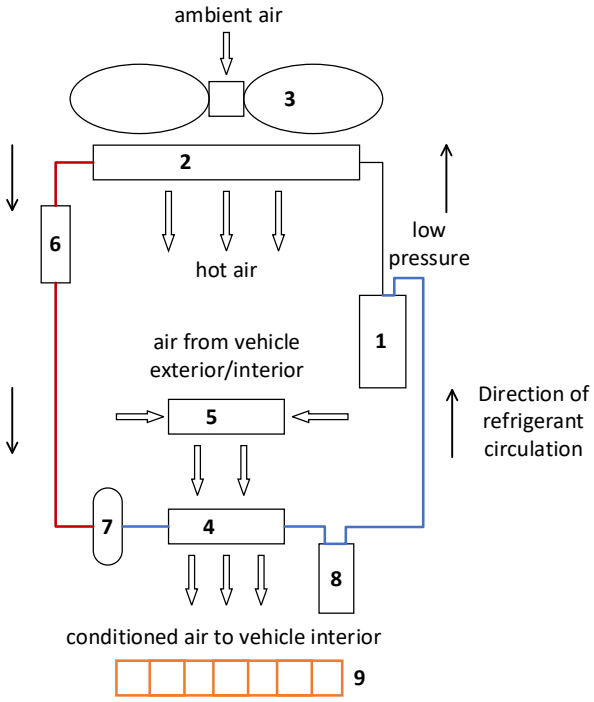

Figure 1 The general overview of automotive air conditioning system. (1 compressor, 2 condenser, 3 fan blowing the air through the condenser, 4 evaporator (cool) 5 blower – pushes the cooled air to the cabin, 6 receiver/driver where the refrigerant is collected, filtered and dried, 7 expansion valve – enables the high-pressure refrigerant to change to low-pressure liquid, 8 suction accumulator, 9 air filter in the cabin of the vehicle)

The air is sucked by the system of fans either from the car exterior or interior and enters the cabin via the air filter to capture the dust, pollen and different atmospheric pollutants. The direction of the air movement through the air conditioning system is showed by the thick arrows in the Figure 1. The thin arrows indicate the circulation of the refrigerating agent via the particular components of the system. It is apparent that the air could come from the car exterior or could be recirculated. The air recirculation mode seems to be the potential source of human

pathogens in the air filters.

1.4. Bus Air Conditioning System

The circulation of the refrigerant and the function of the air conditioning system of the bus is the same as in the case of automotive air conditioning system.

In European countries, the air filters for air conditioning systems follow the ISO 16890 standard. The filters belong to the $PM_{2,5}$ category and are made of non-woven polymeric textile. The buses for municipal public transport usually have a monolayer filter, the transit and coach buses can have pleated filters having more than one layer of filtering media. Some coach buses producers have the filtration unit made of air filter enhanced with UV-C lamp to inactivate potential microbial contamination. In the Northern America the filters fulfil the demands of MERV categorization. Usually, the MERV 7-13 are used. The higher-level MERV 11-13 are typical for transit and coach buses. The MERV standard air filters are usually made of polyester or other polymeric synthetic media. The MERV 13 may contain the electrostatic layer enhancing the collection of charged particles.

When considering the influence and benefit of air conditioning system air filters, the airborne transmission should be taken into regard. Shen at al. reported 24 out of 68 passengers positively tested on SARS CoC 2 after 100 minutes in the coach bus where the air conditioner was set to heating and indoor recirculation(Shen et al. 2021). During the COVID-19 many studies of ground public transport safety were issued Some of them tested the passengers in the coach bus the same way as Shen, some were models of virus transmission. Edwards et al. evaluated COVID-19 control measures including ventilation by opened windows and HVAC system use in the model of school bus and transit bus. In the school bus the ventilation and air circulation provided by opened windows resulted in reductions in the overall particle count, an average of 84% on school bus and 50% on transit bus. When considering use of HVAC with MERV 13 air filter, the effectiveness of removing aerosol particles increased significantly in the transit bus. The resulting particle count with the air filters resulted in an average of 93.95% improvement with aerosols dispersed from a middle location during bus in-motion testing (Edwards et al. 2021).

Zhang et al. investigated potential transmission mechanisms on an urban bus. The bus was fitted with one aerosol generator, to mimic an infected passenger. They identified that the flow carrying aerosols was predominantly controlled by the bus ventilation systems (heativng, ventilation, and air conditioning; HVAC), uniformly distributing aerosol throughout the bus (Zhang

et al. 2021).

Another study made during the COVID-19 pandemic in Italy studied SARS CoV-2 presence in the air and on frequently touched spots of a trolleybus. The transmission mitigation strategies were in place, including increased cleaning, face masks, social distancing and hand hygiene. All samples were tested using specific real-time reverse transcriptase-polymerase chain reaction (RT-PCR). All samples were reported SARS CoV-2 negative. In two weeks of testing, 1100 people travelled by the trolleybus. There was no information if the HVAC system was set on (Di Carlo et al. 2020).

The study from Barcelona, Spain included samples from buses and subway - eighty-two (58 surface swabs, 9 air conditioning (a/c) filters, 3 a/c dust, 12 ambient air. Using an RT-PCR technique for SARS-CoV-2, thirty samples (36%) had evidence for at least one of the three tested viral RNA targets. Interestingly, the surfaces were more contaminated than the air. In addition, there were higher concentrations of viral RNA in buses compared to trains (Moreno et al. 2021).

Some studies focused on infection transmission in the buses, both urban od transit, show different results taking in regard air conditioning system. The urban public transport has a great advantage represented by fast passengers exchange, many passengers stops with door opening which contributes to air enhanced air circulation. The air quality control could be an issue for transit and coach buses which imitate more an enclosed air-conditioned space with a significant role of HVAC in airborne infection transmission.

The study of Lee et al. investigated the influence of air purification system on the indoor air in intercity buses in Korea. They compared concentrations of particulate matter ($PM_{2.5}$ and PM_{10}), airborne bacteria, and carbon dioxide (CO_2) in six buses (three with air purification systems and three without) along three bus routes. The air purifier was installed to the bus additionally. They were equipped by HEPA filters including diodes emitting UV light (UV-LEDs). Using aerosol monitors, the research group showed 34–60% and 25–61% lower average concentrations of $PM_{2.5}$ and PM_{10}, respectively. In addition, buses with air purification systems had 24–78% lower average airborne bacteria concentrations compared to the buses without any air purification (Lee et al. 2022).

1.5. Portable Air Purifiers

In general, commercially available portable air purifier is a simple device driven by an electromotor. The ventilator sucks the air to the apparatus where there is situated mechanical air filter. The filters may be made of vide variety of

material (glass fiber, synthetic polymers or natural fibers). Most commonly HEPA filter represents the best option. The HEPA filters – HEPA 13 and 14 usually must be compatible with the demands of ISO 1822 standard. Some air purifiers include ionization unit which helps to inactivate microorganisms by ionization of the air leaving the device. Other potential ways of air filtration in air purifiers are represented by cold plasma generators, photocatalytic air-cleansing systems and UV-C radiation using wavelength ranging from 200 to 280 nm.

The COVID-19 disease pandemic had intensive influence on indoor air quality level. The air purifiers were widely used in effort to reduce SARS CoV-2 spread. The portable air purifiers were installed to the outpatient departments or tested in the classroom of music school. The music schools represented a significant challenge for indoor air quality during COVID-19 pandemic, especially when regarding the wind-instrument classes, without any possibility of face mask wearing. The proper use of portable air purifiers including optimal placement of the device significantly decreased the aerosols concentrations in experimental music classroom (Narayanan and Yang 2021).

Air purifiers were found useful at school classrooms despite the disadvantages of making noise or having different parameters of air flow. Shulz et al. have made experiments in school classrooms with students and in classroom with aerosol generator. They showed that the use of air purifier leads to a substantial reduction of aerosol particles in the particle size range of 0.178–17.78 μm. These dimensions cover majority of bioaerosols, so the air purifiers could be optimal measure for airborne infection particles reduction (Duill et al. 2021).

In hospitals, the air purifiers can contribute to bioaerosols reduction especially in outpatient departments or in the areas with fast patients' exchange. The simulations with aerosol generator (a person infected with SARS CoV-2) and aerosol detector (a subject with potential contact with SARS CoV-2) concludes that the correct use of PAC can reduce the clearance half-life of aerosols by 82% compared to the same indoor-environment without any ventilation, and at a broadly equivalent rate to built-in mechanical ventilation (Salmonsmith et al. 2023).

The portable air purifiers had been recommended by US Environmental protection agency for homes as well to reduce the risk of SARS CoV-2 transmission (The United States Environmental Protection Agency 2023) Regarding all indoor areas, the air purifiers are capable to reduce bioaerosols concentration, improve the indoor quality, but using as a single preventive measure are not sufficient enough. It is necessary to keep good ventilation, air exchange (Salmonsmith et al.

2023) and sanitation of indoor equipment as well (Christopherson et al. 2020).

1.6. Microbial Contamination of the Air Filters

1.6.1. Bacterial Contamination

The microorganisms could enter the air conditioning system from two main sources, the external atmosphere and human microbiota. The exterior atmosphere contains the ubiquitous microbes present naturally in the air, dust, soil or plants. Especially the bacteria present in such milieu are usually very resistant and can contaminate the air filters very easily.

The bacteria of the species *Bacillus* and *Brevibacillus* are especially present in the soil and dust. The most common are *Bacillus cereus*, *Bacillus subtilis*, *Bacillus licheniformis*, *Brevibacillus* brevis and *Brevibacillus laterosporus*. The soil contains also *Clostridium tetani*, *Clostridium perfringens* and *Clostridium botulinum*. Presence of these bacteria in the environment can represent the health risk under particular conditions. The *Bacilli* and *Brevibacilli* are the facultative pathogens in humans with compromised immune system, only *Bacillus anthracis* threatens all people. The abovementioned *Clostridia* are obligatory pathogens for people. The genera *Flavobacterium*, *Methylobacter*, *Sphingomonas* are also naturally present in the atmosphere but do not cause disease in humans. The soil could contain a wide variety of different bacteria – especially *Actinomyces* or *Flavobacterium* or *Nocardia*. The soil microbiota could not be complete without presence of fungi of the genera *Aspergillus* and *Penicillium*. *Aspergillus niger* is ubiquitous, *Aspergillus fumigatus* could represent a health risk for people with immunity disorders.

More interesting and more important could be the human microbiota as a source of microbial contamination of the air filters in the output of car air conditioning system. The microorganisms coming from the passengers' mouth, skin or gastrointestinal tract could contaminate the air filter mainly when recirculation mode is switched on.

The mouth microbiota contains hundreds of microorganisms especially the members of genera *Streptococcus, Neisseria, Staphylococcus* or *Micrococcus*. We can also find *Actinomyces*, sometimes *Candida*. The *Streptococci* are the most abundant, their function is mostly protective, but mainly *S. mutans* cooperates in caries production. *S. sanguis* or *S. mitis* could represent a potential health risk for people with disorders of the heart, blood vessels or heart valves causing endocarditis, less often bacteriemia or sepsis in susceptible persons (Goering et al. 2016). Some people could also have *Staphylococcus aureus* or *Neisseria meningitidis* present in the mouth or oropharynx.

The anaerobic bacteria make a significant part of human oral microbiota as well. The members of the genera *Propionibacterium, Veillonella, Peptococcus, Lactobacillus or Bacteroides and Fusobacterium* or *Bifidobacterium* are very important in mouth mucosa protection. The balanced oral microbiota protects the oral mucous membrane and teeth with periodontal tissues from attacks of pathogens and other microorganisms which could cause a serious microbiota disbalance or some particular disease. The oral microorganisms live usually in biofilm. Despite the tight adherence of the microorganisms in the biofilms, they could be expelled to the external environment during speaking, cough or sneezing. Then they could be trapped by the cabin air filters of the car air conditioning system.

The same situation can occur in the case of the members of the skin microbiota. There we can usually find mainly *Staphylococcus epidermidis* and *Propionibacterium acnes*. The members of genera *Corynebacterium* or *Micrococcus* and *Streptococcus* also belong to the skin microflora (Skowron et al. 2021). The commensals have essential protective function. Only under special condition, they could contribute to disease development.

The richest but in this case perhaps less important is the gastrointestinal tract microbiota. It cannot be omitted to complete all potential air filter contaminants. *Enterobacteriaceae*, *Clostridiaceae*, *Peptostreptococcaceae* and *Enterococcaceae* are the bacterial families present in large amount in the intestine (Murray, Rosenthal, and Pfaller 2016). Most bacteria belong to the genera *Bacteroides*, *Clostridium*, *Faecalibacterium*, *Eubacterium*, *Ruminococcus*, *Peptococcus*, *Peptostreptococcus*, and *Bifidobacterium*. Other genera, such as *Escherichia* and *Lactobacillus*, are present to a lesser extent (Guarner and Malagelada 2003). In healthy persons the bacteria proliferating in the intestines could contaminate the hands and then the microorganisms could be released from the skin to the air and potentially contaminate the air filters.

1.6.2. Respiratory Viruses as Contaminants of the Air Filters

We can expect contamination of air filters by respiratory viruses to wide extent. As mentioned above, Influenza A and Influenza B viruses was detected on air filters in the commercial aircraft. Parainfluenza viruses belong to potential contaminants as well (Goyal et al. 2011). Rhinovirus was detected in indoor air of office building equipped with heating ventilation air conditioning system (HVAC) (Myatt et al. 2004), so the contamination of air filter could be expected. The influenza or common cold infections are usually limited to cool months of the year (Fisman 2012). The respiratory syncytial virus (RSV) was proven in HVAC

filter in children's day care center in seasonal manner (Prussin et al. 2016). Adenoviruses represent another representants of airborne infectious agents. Under experimental conditions, most aerosolized adenovirus particles were stopped by fiberglass air filter in air handling unit (AHU) tests, but several viral particles penetrated the filter with negligible loss of infectivity (Bandaly et al. 2019). Recently, the severe acute respiratory syndrome virus-2 SARS CoV-2 could be present on the air filters which are used to clean the indoor air (Nazarenko 2020). The SARS-CoV-2 is RNA virus, enveloped and belongs to rather smaller viruses. The SARS-CoV-2 occurs through respiratory droplets, droplet nuclei or virus aggregates (Kampf et al. 2020). The droplet nuclei and aggregates are important for air filtration because the droplets sediment quickly within two meters far from infected individuals (Heo, et al., 2021). SARS-CoV-2 in the form of aerosolized particles, which are found in a spectrum of sizes, typically 0.25 to 0.5 μm, nearly resembles the MMPs diameter. In fact, the HEPA filters are the most important air filters in virus removal, even in cleanrooms (Sandle 2020). Some studies suggest the portable air purifiers as adjunctive infection control measures with knowledge of HEPA filter functionality and limitations in mind (Christopherson et al. 2020).

1.7. Air Filters

Quality and arrangement of air filters play the key role in the air cleanliness. Currently a wide variety of filters are available. There are several categories of filters suitable for particulate filtration – mechanical and electrostatic. The mechanical filters can be also enhanced with activated carbon to remove vapors, smells and volatile organic species VOCs.

Theoretical explanation of particles behavior is dependent on particle-fiber interaction within the filtration fibrous material. The mechanisms of air filtration in fiber-based filtration medium are shown in the Figure 2.

1.8. Nanomaterial and Nanotextile in Air Filtration

Recently, nanotechnology field created high impact in various spheres such as healthcare or environment – especially for gaseous and particle pollutant capture (Ravichandran et al. 2012), (Orlando et al. 2021).

Many different materials can be processed by electrospinning to produce nanofibers. Among organic polymers are leading materials polyamide PA6/12, Nylon PA6 or Polyaramid Al_2O_3, then polyurethan PUR and polyvinyl alcohol PVA could be used for nanofiber fabrication (Lev, Kalhotka, and Cerný 2010). The filtration efficiency of Nylon6 nanofiber material and high-efficiency air

particle filter (test particles with an air flow speed of 5cm/s and 0.3μm) was compared. The results showed that the filtration efficiency of nano-fiber mat can reach 99.993%, which is much higher than that of high-efficiency air particle filter (Ahn et al. 2006). Polyacrylonitrile-based nanofibers (PAN) could be suitable for air filtration application as well (Nataraj, Yang, and Aminabhavi 2012). Electrospun nanofiber materials have high-efficiency filtration performance, but nanofibers can withstand low strength, are very fragile, easily damaged, and have extremely poor durability. Therefore, nanofiber mats cannot be used as filters alone. In order to apply nanofibers to filtration, it is necessary to compound the nanofibers with the base fabric to increase their mechanical strength. Podgórski et al. suggested triple layer design for removing nanoparticles along with other aerosol particles. The first layer is a porous microfiber-based media for collecting the micro particles, middle one is the nano-fibrous membrane for capturing the particles in between 100 and 500 nm and the back layer is the dense layer of microfibers to provide mechanical strength to the composite media (Podgórski, Bałazy, and Gradoń 2006). Patanaik et al., prepared three-layered composite fiber filter media (the middle layer is electrospun nanofibers, and the front and back layers are nonwoven base fabrics) and double-layered fiber composite filter media. The nanofibers are deposited on the nonwoven base fabric, and the durability of the nanofiber layer is tested by circulating compressed air through these two filter media. The pore size of the layer is significantly increased, resulting in a significant change in filtration efficiency and pressure drop. For three-layer fiber composite filter media, there is no significant change in pore size, filtration efficiency (Patanaik, Jacobs, and Anandjiwala 2010).

2. Aim of the Work

Existing air filters usually placed to air conditioning systems usually have capacity to collect particulate matter 0,3-10 (PM) μm in diameter. But some of bacterial pathogens and most viruses pathogenic for humans are incomparably smaller. This is the true reason why the verification of air filter's efficiency is necessary.

The work has several parts and is mainly aimed at:

1) Tests of bacterial contamination of selected air filters will be performed to find and identify exactly particular pathogens present on the inlet and outlet surfaces of the filter. The special emphasis will be given to the evaluation of the microorganism burden of the

outlet surface of the filters to assess the efficiency of the filters.

2) The nanomaterial efficiency in capturing microorganisms will be studied. The specimen of patented nanotextile will be inserted to the household air purifiers as a model air filtration device equipped with the HEPA filter and the swabs and microbiological tests of microorganisms present on the surfaces of nanotextile and HEPA filter will be carried out.

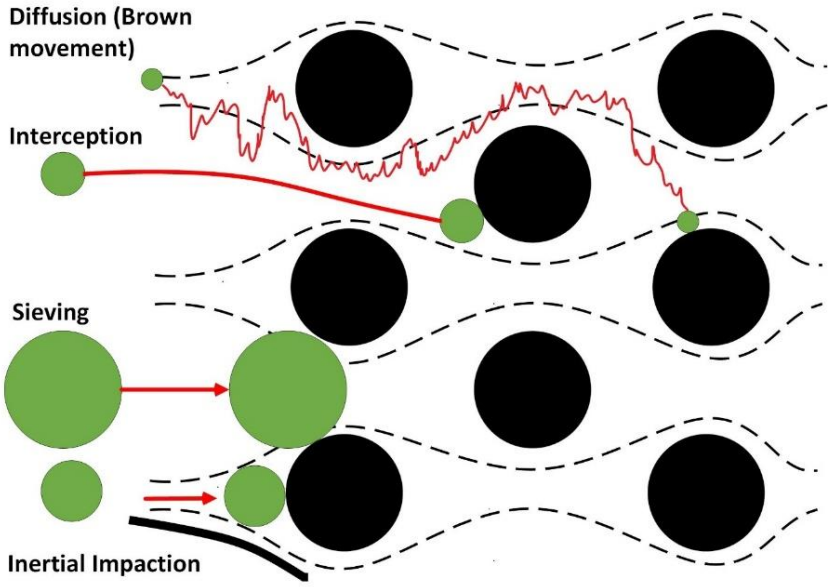

Figure 2 Filtration mechanisms. The figure represents a cross section of a fiber-based filtration medium. The interrupted black line suggests the direction of air flow. The black circles are the cross sected fibers of the filtration medium. The green objects show filtered particles. The red lines or arrows demonstrate the direction of filtered particles (Daniela Obitková 2023). The detailed description of individual collecting mechanisms is as follows: Inertial impaction works usually on large and heavy particles, usually larger than 1 micrometer in diameter. They are heavier than the air passing along the media fibers. As the air changes direction to get round the media fiber, the large particle collides with that fiber and is captured and held.

- On the other hand, the diffusion is the mechanism of capturing of the smallest particles. This kind of particles traverse the viscous flow of the air in the filtration media and interacts with the fibers and are collected.
- Mid-range size particles are captured by direct interception. As the viscous flow enters the filtration media, the flow bends along the fibers and the particles are trapped directly.
- Sieving, the most common mechanism infiltration, occurs when the particle is too large to fit between the fiber spaces (Donaldson Company Inc), (National institute of Occupational Safety and Health 2003).

3. Material and Methods

All methods employed in this work correspond with standardized methods accredited in microbiology. The material and equipment both disposable and reusable used to fulfil the requirements of this work are available via registered manufacturers or suppliers.

3.1. Chemicals

Chemicals were obtained from (P-Lab, CZ, Penta, CZ) - gram stain kit Carl Roth, acetone, ethyl alcohol. Sterile saline solution (0,9% NaCl Braun, Germany) served as a sampling solution. MALDI matrice alpha-Cyano-4-hydroxycinnamic acid (Biovendor CZ), dilution solution Bruker standard solvent (Merck CZ) were used for mass spectrometry. To avoid any undesirable contamination by DNA, Termi-DNA-tor spray (Dynex, CZ) was used during sampling for obtaining the specimen of nucleic acids.

3.2. Material and Instrumentation

The dry swabs were taken by polyester swabs with plastic shaft packed individually (Inset Ltd. CZ), Then the cultivation was performed on standard solid cultivation plates – Petri plates (diameter 9 cm) – blood agar, blood agar with 5% NaCl, Mueller-Hinton, Sabouraud, Endo, McConkey agars (Biovendor CZ). All samples' manipulations were performed in biohazard box BSL 2 (Schoeller CZ). For cultivation, the thermostat Memmert (Merci CZ) with precise temperature regulation was used. For anaerobic cultivation, the anaerostat Oxoid with Anaerocult® bags (Merck Germany) were employed. Small disposable material like glass microscopic plates, plastic pipettes and pipette tips were supplied by P-Lab CZ. The immerse microscopy with 100x objective was performed with the microscope Labomed 400 (Swen Biolabs CZ).

MALDI-TOF To precise bacterial identification, the Bruker MALDI TOF Biotyper (Germany) mass spectrometer was employed.

The nucleic acid research was performed with The BioFire® FilmArray® 2.0 System (Biomerieux CZ). The fully automated system uses panels for multiplex PCR search. The Respiratory and Pulmonary panels were used.

As a second multiplex PCR system The QIAStat DX® Analyzer 1.0 with the Respiratory SARS CoV-2 Panel (Genetica Ltd, CZ) a fully automated device used for multiplex RT PCR analyses was used.

3.3. Air Filter Specification

The air filters from different air conditioning systems and air conditioning devices were chosen. Majority of the air filters come from the air conditioning systems of the means of transport:

- Aircraft air filter. The first filter is micropore filter of cylindric shape, with hollow interior. The material made of glass fibers is pleated inside metallic skeleton. Recommended flying hours are 5000 h. The filter was removed during regular service from Airbus A319 operating only European destinations.
- Automotive air filter. Automotive air conditioning air filters made of glass fiber material (SO 16890 $PM_{2,5}$) boosted with anti-pollen layer were removed from cars during regular service – after 1 year or after 20 000 km of operation.
- Buss air conditioning filters. The bus air conditioning system in the passengers' cabin is filled by polyethylene or polyester filters (ISO 16890 $PM_{2,5}$). The regular service interval does not exceed 2,5 months in the warm season of the year.
- Household air purifier. A household portable air purifier produced in the Czech Republic equipped with standard HEPA filter (HEPA 13, EN 1822-1) was used as a model devicefor our tests and experiment. The chosen air purifier was purchased in the retail chain and is suitable for household use only. During the experiment we used the maximal airflow 145m^3/h, the possibility of air ionization was switched off. The device was placed in the room having floor space 63m^2 and 138.6 m^3 volume of the ai. The experiment was performed during spring season 2019 and 2021 at room temperature ranging from 20 °C to 25 °C.
- Nanotextile material. The nanotextile specimen was obtained from the Nanotex Ltd. Czech Republic. The substance of the nanomaterial is polyamide 6 (PA6) with porosity of 70 nm and fiber thickness 100-500 nm. The thermo-resistance achieves to 126 °C. Nanotextile itself is fixed to polyester non-woven fabric.

3.4. Bacteriological Techniques

The ISO EN 18593 Microbiology of the food chain - Horizontal methods for surface sampling was taken as a basis for sampling the air filters ("ČSN ISO EN 18593: Mikrobiologie potravinového řetězce - Horizontální metody specifikující techniky vzorkování z povrchů" 2019). The standard specifies the horizontal swab

techniques which could be simply used for air filters lamellas sampling. Especially the swab technique specification gives applicable advice on how to obtain reasonable samples for microbiological investigation.

All filters were swabbed by dry polyester swabs – always the inlet surface and the outlet surface separately. The swabbed material was directly inoculated to the solid cultivation media and cultivated at 36±1 °C in aerobic and anaerobic atmosphere for 24 hours. For quantification the swabs obtained from inlet and outlet surfaces were rinsed to sterile saline solution – 1-10 ml. Cultivated bacteria were identified by Gram stain and immerse microscopy (1000x magnification), biochemical tests and by MALDI-TOF mass spectrometry.

3.4.1. Biochemical Tests

The biochemical and metabolic characteristics were tested by a set of biochemical tests - CATALASE test, STAPHYt est STREPTO test, EN-COCCUS test, OXIDASE test, ENTERO test, OXIDATION-FERMENTATION test and CANDIDA test (Erba Lachema CZ).

3.4.2. Standard Plate Count

Integral part of the bacteriological procedures is represented by bacteria quantification. The standard plate count method was performed to enumerate the bacteria, the results are represented in CFU/ml (Erkmen and Erkmen 2021).

The procedure of standard plate count follows:

- Dry swabs of inlet and outlet surface of the filter
- Rinse in the sterile saline solution, volume 1 ml or 10 ml
- If necessary – logarithmic dilution of the saline solution obtained in the previous step
- Inoculation of 200 μl from each dilution to the Petri plate filled with blood agar
- Incubation 24-72 hours, temperature 36± 1 °C
- Choose of the plates where there are 20-200 colonies
- Counting of the number of colonies per plate
- Calculation of CFU per milliliter according the formula:

$$\text{CFU/ml} = \frac{number\ of\ colonies\ per\ plate}{diolution\ factor * number\ of\ plates * inoculated\ volume\ [ml]}$$

3.5. MALDI -TOF Mass Spectrometry

Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) represents an analytical technique which brings diagnostic

accuracy and quick proteome estimation of microorganisms. The technique was chosen due to wide variety of members of genus Bacillus expectation. MALDI TOF technique is precise tool for different Bacilli differentiation. The decision to employ the proteomic technique was also supported by UK Standards for Microbiology Investigations (UK Standards for Microbiology Investigations 2018). The specimens of 24-hours cultures were placed on the MALDI plate with round target places and spread thoroughly. Each target place with bacterial specimen was overlayed with 1μl of MALDI matrix – alpha-Cyano-4-hydroxycinnamic acid (HCCA) diluted in Bruker standard solvent – 50% Acetonitrile, 47.5% purified water, 2.5% Trifluoroacetic acid, net concentration 10mg/ml (Bruker Daltonik GmbH 2010). Dried MALDI Bruker plate was installed to the MALDI-TOF analyzer. The microbiology software automates the process of acquiring the mass spectra. The obtained spectra are matched against the extensive reference library. Then the result is scored. The comparison of the sample and library data gives the number of congruent mass spectrum peaks. The maximum number is 1000, the minimum is 200. The calculation uses logarithmic scale. Log_{10} 1000 = 3, i.e., so the maximum score is 3. The minimum score for reliable detection amounts to 2 and 3.

3.6. Polymerase Chain Reaction Assay (PCR)

Multiplex polymerase chain reaction (PCR) is a variant of PCR in which two or more target sequences can be amplified by including more than one pair of primers in the same reaction mixture. In the field of infectious diseases, multiplex PCR has been shown to be a valuable tool for identification of viruses, bacteria and parasites (Markoulatos, Siafakas, and Moncany 2002).

3.6.1. The BioFire® FilmArray® 2.0 System

The BioFire® FilmArray® 2.0 System is fully automated multiplex PCR instrument designed for syndromic diagnostic use. Using The Respiratory panel, mainly the viruses attacking the upper respiratory tract were searched. The Respiratory panel contains 20 targets listed in the table.

The Respiratory panel was used for microorganism detection in aircraft and automotive air filters, then it was used for the first experiments with HEPA filter and nanotextile from household portable air purifier. The filters or nanotextiles were swabbed by dry polyester swabs, inlet and outlet separately. The swabs were rinsed to the sterile saline, 1 ml, to produce sample solution. 300 μl of this sample solution was applied to the sample section of the cartridge, then the sample buffer was added to fill up the sample section of the cartridge. As the second step, the hydration buffer was added to the particular section of the cartridge. The cartridge

of Respiratory panel, sample buffer and hydration buffer are supplied by the producer ready to use. The list of targeted pathogens in Respiratory panel is listed in the Table 3.

Table 3 The list of pathogenic agents included in BioFire® FilmArray® Respiratory panel

Pathogenic agent	Classification
Adenovirus (AdV)	Adenovirus (DNA)
Coronavirus (CoV) 229E, HKU1, NL63, OC43	Coronavirus (RNA)
Enterovirus (EV)	Picornavirus (RNA)
Human Rhinovirus (HRV)	
Human Metapneumovirus (hMPV)	Paramyxovirus (RNA)
Influenza A (Flu A) (subtypes H1, H1-2009, and H3)	Orthomyxovirus (RNA)
Influenza B (Flu B)	
Parainfluenza Virus 1 (PIV1)	Paramyxovirus (RNA)
Parainfluenza Virus 2 (PIV2)	
Parainfluenza Virus 3 (PIV3)	
Parainfluenza Virus 4 (PIV4)	
Respiratory Syncytial Virus (RSV)	
Bordetella pertussis	Bacterium (DNA)
Chlamydophila pneumoniae	
Mycoplasma pneumoniae	

The Pulmonary panel targets contains mainly bacteria - *Acinetobacter calcoaceticus-baumannii* complex, *Enterobacter cloacae* complex, *Escherichia coli, Haemophilus influenzae*, *Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae* group, *Moraxella catarrhalis*, *Proteus* spp., *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes.* Atypical bacteria*: Chlamydia pneumoniae*, *Legionella pneumophila*, *Mycoplasma pneumoniae.* Viruses are listed in following Table 4.

Table 4 The list of pathogenic agents included in Pulmonary panel

Pathogenic agent	Classification
Adenovirus (AdV)	Adenovirus (DNA)
Coronavirus (CoV)	Coronavirus (RNA)

Human Metapneumovirus (hMPV)	Paramyxovirus (RNA)
Enterovirus (EV)	Picornavirus (RNA)
Human Rhinovirus (HRV)	
Influenza A (Flu A)	Orthomyxovirus (RNA)
Influenza B (Flu B)	
Parainfluenza Virus (PIV)	Paramyxovirus (RNA)
Respiratory Syncytial Virus (RSV)	

The Pneumonia panel contains also the antimicrobial agents resistance genes: carbapenemase genes, ESBL genes and the target genes of resistance to Methicilline. The exact list of antimicrobial resistance genes is summarized in the Table 5.

Table 5 The list of antimicrobial agents resistance genes

ESBL genes	Carbapenemases genes	Methicilin Resistance genes
CTX-M	KPC	mecA/mecC and MREJ
	NDM	
	Oxa48-like	
	VIM	
	IMP	

The work procedure is the same as in Respiratory panel. The amount of sample solution was 200 μl. The Pulmonary panel was used in the case of the first experiment with the nanomaterial applied to the household portable air purifier.

The results of the Respiratory panel were released as qualitative mode – detected/not detected. The Pulmonary panel enables qualitative results representation and also semiquantitative results presentation. The qualitative result presentation is depicted in the Figure 3, where the peak of fluorescence of detected microorganism is visible. There is the melting temperature on the x axis, the y axis represents the intensity of fluorescence. The chart was obtained as a print screen of the BioFire® FilmArray® 2.0 System. Thew semiquantitative result representation means, that the PCR procedure is capable to quantify numbers of nucleic acid copies per volume of the sample.

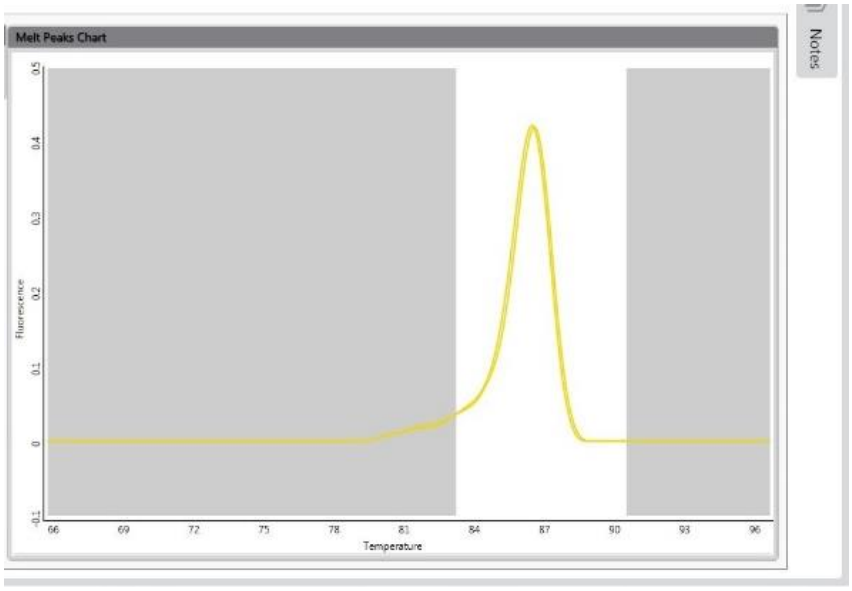

Figure 3 The peak of Seratia marcescens obtained by FilmArray 2.0, Pulmonary panel (Daniela Obitková 2019)

3.6.2. QIAStat DX® Analyzer 1.0

The multiplex PCR fully automated system designed mainly for clinical syndromic testing enables detection of multiple targets in a one run. The chosen Respiratory panel is supplied in single-packed cartridges containing all reagents for sample preparation. The reagents include the internal controls as well. The cartridges provide two ways of sample preparation. The flocked swab supplied by the producer of the QIAStat DX® Analyzer 1.0 can be inserted to the cartridge as whole to the inlet opening. The second way of sample preparation is recommended for liquid samples (sputum, lavage). As recommended by the producer, 200 μl of liquid sample is suitable for the analysis. Each sample undergoes one run in duration of 60 minutes. The results are released in qualitative mode – detected/not detected.

The chosen air filters (automotive, nanomaterial monolayers) were swabbed by dry polyester swabs, inlet and outlet surface separately. Each swab was rinsed in 1 ml of sterile saline solution (sample solution). To the cartridge, 200 μl of sample solution was injected to the inlet chamber. The complete list of Respiratory Panel targets is summarized in the Table 6.

Table 6 The Respiratory SARS CoV-2 Panel (Producers' manual to the Respiratory panel)

Pathogenic agent	Classification
Influenza A	

Influenza A (subtype H1N1/2009)	Orthomyxovirus (RNA)
Influenza A (subtype H1)	
Influenza A (subtype H3)	
Influenza B	
Coronavirus 229E	Coronavirus (RNA)
Coronavirus HKU1	
Coronavirus NL63	
Coronavirus OC43	
SARS-CoV-2	
Parainfluenza virus 1	Paramyxovirus (RNA)
Parainfluenza virus 2	
Parainfluenza virus 3	
Parainfluenza virus 4	
Respiratory syncytial virus A/B	
Human Metapneumovirus A/B	
Adenovirus	Adenovirus (DNA)
Bocavirus	Parvovirus (DNA)
Rhinovirus/Enterovirus	Picornavirus (RNA)
Mycoplasma pneumoniae	Bacteria (DNA)
Legionella pneumophilla	
Bordetella pertussis	

4. Results

Four kinds of air filters underwent the study. The complete list of investigated air filters including the nanotextile used as an experimental model are listed in the Table 7. At first the standard bacteriological procedures were employed for detection and identification of diverse bacterial contamination of aircraft air filter. To fulfill the current requirements on accuracy and precise pathogens identification the techniques of molecular biology were employed represented by multiplex PCR (polymerase chain reaction) procedure. Multiplex PCR was used for detection of microbial contamination in all three kinds of filters included to the research. All tests of aircraft filter were made in cooperation with Bc. Viktoriya Gvozdeva (Gvozdeva 2018) and Ing. Milan Mráz.

Table 7 Filters included to the study.

Sort of the filter	Source of the filter

aircraft air filter	Airbus A 319
Automotive air filters	Private cars and pick ups
Bus passengers' cabin air filters	City buses Czech Republic
HEPA filter	household portable air purifier
nanotextile 1	household portable air purifier
HEPA filter 2	household portable air purifier
Nanotextile 2,3,4	household portable air purifier

4.1. Aircraft Air Filter

The air filter was removed aseptically during the regular service of air conditioning system in authorized service center. The filter served more than 4800 flying hours. Avoiding the contamination, the filters were transported in plastic covering to the laboratory and treated in laminar-flow class II biosafety cabinet. In addition, all the principles of sterile work and personal safety were followed.

Figure 4 The air filter removed from air conditioning system of Airbus A319 (Daniela Obitková 2019)

The filter has the shape of hollow cylinder having metallic framework resembling the thin web covering the surface of the filtration lamellas folded in transverse axis. In the Figure 4, in the left part of the picture, there is the inlet surface of the filter. In the right part of the picture there is presented the insight to the cavity of the filter where the outlet surface of the filter is situated. The filter we investigated was enriched with charcoal powder placed deeply in between the lamellas. The filter came from Airbus A319 flying within European routes.

4.1.1. Sampling

Horizontal sampling technique was adopted from the ISO 18593. Both dry and damp swabs were obtained. When considering the material of the swabs, the polyester material Dacron was evaluated as the best. They are suitable for taking samples from difficult available places with thin slots and in dry state, it serves as an efficient brush providing optimal swab yield. At first, the squares having the

edges about 15 cm long were marked on both inlet and extract filter surface. The swabs were taken from the surface of the lamellas within the square. Moreover, the charcoal contamination of the swabs should have been avoided. About 100 swabs were taken from both extract and inlet surface of the filter. The swabs were inoculated to culture media.

To obtain the most efficient viral samples, the Microtest TM M4RT® Kit: Swabs: 2 Female (Remel Europe Ltd) suitable for virus samples taking and transport was used.

4.1.2. Cultivation

Liquid and solid cultivation media prepared at the Institute of Immunology and Microbiology of the 1st Medical Faculty, Charles University were used to cultivate diverse bacterial species. Namely peptone broth, blood agar, Mueller-Hinton agar, Endo's medium and deoxycholate-citrate, Endo agar. For fungi cultivation, the Sabouraud's agar was used. 24 hours cultures were prepared in thermostat maintaining the temperature of 37 °C ± 1 °C.

4.1.3. Qualitative Evaluation of Bacterial Contamination of the Filter

Specimens of pure cultures were treated with Gram's stain. The microscopes Olympus CX 23were used to identify the bacterial species. The microscope BMS Eduled with Bioview camera provided the recording of photographs using the BMS software.

Table 8 G+ bacteria swabbed from inlet and extract side of the filter

Bacterial species	Inlet side	Outlet side
Staphylococcus aureus	NO	YES
Streptococcus pyogenes	YES	YES
Streptococcus pneumoniae	NO	YES
Enterococcus faecalis	YES	NO
Clostridium tetani	NO	YES
Clostridium perfringens	NO	YES

Table 9 G- bacteria swabbed from inlet and extract side of the filter

Bacterium species	Inlet side	Outlet side
Escherichia coli	YES	YES
Citrobacter spp.	YES	YES

Yersinia spp.	NO	YES
Yersinia pseudotuberculosis	YES	YES
Morganella morgani	YES	NO
Klebsiella ozeanae	NO	YES
Pseudomonas aeruginosa	NO	YES

For accurate identification of bacterial species the commercially available microbiological tests were employed – ENTEROtest 24, STREPtest, OXItest (ErbaLachema), ProlexTM staph Latex Kit (Prolab Diagnostics).

Isolated and identified bacteria are listed in Table 8 and Table 9.

4.1.4. Quantitative Comparison of Bacteria Detected on Both Filter Sides

Four bacterial species were chosen for quantification. The standard bacterial plate count was provided to quantify the bacterial contamination of both surfaces of the filter using CFU/ml unit.

The main principle of this technique means that one viable bacterium can produce one colony. The specimen of bacterial suspension is diluted logarithmically obtaining appropriate aliquots diluted 100x (10-1) to 10000000 (10-7). So, seven aliquots with dilution factor 10-1 to 10-7 were prepared. Specimens of selected diluted aliquots are inoculated to Petri plates with solid medium and cultivated for 24-36 hours. Three plates of each selected aliquots are cultivated. After cultivation the plates having 20-200 colonies were taken. The colony number was counted precisely to have the average result of each triplet of plates with selected dilution factor. Then the calculation was carried out. The result of quantification of selected bacteria is listed in the Table 10. It is clearly visible that the outlet surface is ten times more contaminated than the inlet surface of the filter.

Table 10 Quantitative comparison of bacteria detected on inlet and extract sides of the filter.

Bacterium species	Inlet side	Outlet side
	CFU/ml	CFU/ml
Escherichia coli	$3{,}8 \times 10^6$	$1{,}56 \times 10^7$
Yersinia pseudotuberculosis	$4{,}1 \times 10^6$	$1{,}74 \times 10^7$
Citrobacter spp.	$3{,}2 \times 10^6$	$1{,}63 \times 10^7$

4.2. Automotive Air Filters

Automotive air filters were obtained in a car service during regular

guaranteed inspection. The filters served for one year or 20 000 driven kilometers. The filters were removed aseptically, packed in plastic bags, transported to the laboratory where they were processed. Maximum time of storage at laboratory temperature was 2 months. Six filters underwent bacteriological study and the viruses search. In the Table 11, the dimensions of air filters are listed. The table also contains the size in square centimeters.

Table 11 The dimensions and area of selected air filters

filter number	height (cm)	width (cm)	area (cm^2)
11	50	10	500
16	24	19.5	468
17	23	20.5	471.5
19	25	20	500
20	25	20	500
21	25	20	500

The cultivation and immersion microscopy revealed mainly three species of the genus *Bacillus*. We detected *Bacillus cereus, Bacillus subtilis* and *Bacillus licheniformis* on all the filters. The Figure 5 represents the cultivated *Bacilli* on blood agar plates. In the filter 17, on the inlet side *Brevibacillus laterosporus* was identified as well.

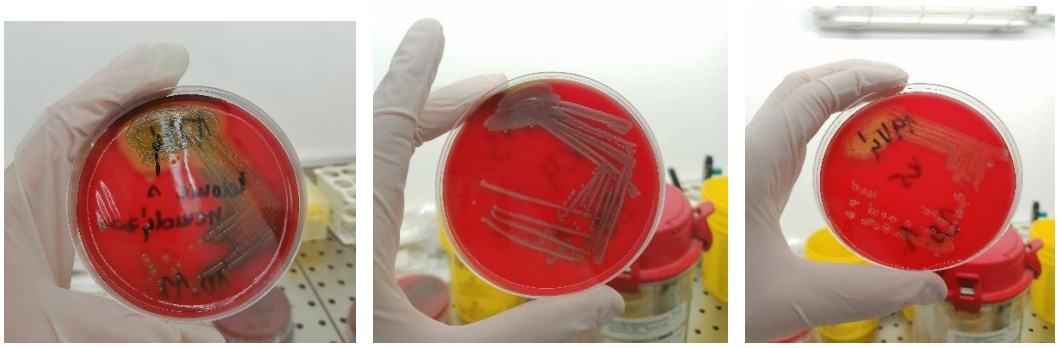

Figure 5 Blood agar plates with 24-hours cultures of detected bacteria. The first picture refers to the culture of *Bacillus cereus*, in the middle the culture of *Bacillus subtilis* is situated and on the right side the culture of *Bacillus licheniformis* is depicted (Daniela Obitková 2021)

In the next section, the tables summarize the bacterial contamination of the filters number 11, 16, 17, 19, 20 and 21 in CFU/ml. The dry swabs of each surface were rinsed to the sterile saline solution, 1 ml. This prepared sample was not diluted, 100 μl was directly inoculated to the blood agar and cultivated 24-72 hours in the temperature 36 ± 1 °C. The Tables 18-23 show the results of quantification of automotive air filters.

Table 12 Filter no 11

	CFU/ml inlet surface	CFU/ml/cm² inlet surface	CFU/ml outlet surface	CFU/ml/cm² outlet surface
Bacillus cereus	6	0.012	0	0
Bacillus subtilis	1	0.002	1	0.002
Bacillus licheniformis	6	0.012	0	0

Table 13 Filter no 16

	CFU/ml inlet surface	CFU/ml/cm² inlet surface	CFU/ml outlet surface	CFU/ml/cm² outlet surface
Bacillus cereus	2	0.0042	0	0
Bacillus subtilis	3	0.0064	1	0.0021
Bacillus licheniformis	2	0.0042	2	0.0042

Table 14 Filter no 17

	CFU/ml inlet surface	CFU/ml/cm² inlet surface	CFU/ml outlet surface	CFU/ml/cm² outlet surface
Bacillus cereus	2	0.0042	1	0.0021
Bacillus subtilis	4	0.0084	0	0
Bacillus licheniformis	5	0.01	0	0

Table 15 Filter no 19

	CFU /ml inlet surface	CFU/ml/cm² inlet surface	CFU/ml outlet surface	CFU/ml/cm² outlet surface
Bacillus cereus	11	0.022	1	0.002
Bacillus subtilis	5	0.01	3	0.006

Bacillus licheniformis	16	0.032	3	0.006

Table 16 Filter no 20

	CFU/ml inlet surface	CFU/ml/cm² inlet surface	CFU/ml outlet surface	CFU/ml/cm² outlet surface
Bacillus cereus	3	0.006	1	0.002
Bacillus subtilis	5	0.01	1	0.002
Bacillus licheniformis	9	0.018	2	0.004

Table 17 Filter no 21

	CFU/ml inlet surface	CFU/ml/cm² inlet surface	CFU/ml outlet surface	CFU/ml/cm² outlet surface
Bacillus cereus	2	0.004	0	0
Bacillus subtilis	4	0.008	0	0

4.2.1. MALDI TOF Proteomic Study

The specimens of 24-hours cultures were placed on the target plate with preceding formic acid treatment. Then 1 μl of matrix was used to superimpose the specimen. After getting dry, the target plate was placed in the cell of the Bruker instrument. The colonies which were not identified reliably underwent proteomic study. The Table 18 summarizes the results of proteomic study. *Brevibacillus borstelensis* occurred only on the inlet surface, *Bacillus clausii* was detected on the outlet surface of the filter 16. On the outlet surface of the filter 17, *Bacillus fordii* was identified. Their amount was not quantified due to presence only on the single surface of the filter. The results of MALDI TOF bacteria identification are presented in the Table 18.

Table 18 The proteomic study results.

Filter number	Detected microorganism	Score value
Filter 16 inlet surface	*Brevibacillus borstelensis*	2.40

Filter 16 outlet surface	*Bacillus clausii*	2.03
Filter 17 outlet surface	*Bacillus fordii*	2.19

The contamination of the filters by environmental bacteria is low, but the results show that the different members of the genus bacilli which have larger diameter can penetrate the filter media. Interestingly, the *Bacillus clausii* and *Bacillus fordi* were detected only on the outlet surfaces of the filter 16 and 17 respectively.

4.3. Bus Air Conditioning Air Filters

The number of five filters from bus passengers' cabin air conditioning system was investigated. The busses served in city public transportation in the Czech Republic. The service intervals for filter exchange are as follows – the new filter is installed after winter pause in May, then the exchange comes in July and the last change of the filter is performed at the end of September. The filters for the investigation were removed in July and September. The filters are made of polyester non-woven textile supplied as footage 12 mm of width. The filter material meets the requirements of ISO 16890 $PM_{2,5}$. This kind of filter captures particles of diameter 0,3-2,5 μm with 50% effectiveness. The city buses of investigated specimen have the air conditioning unit situated at the rooftop. The cooling medium is driven by the compressor connected to the motor of the bus. The evaporator is situated at the rooftop as well. The hot air is sucked from the cabin of the bus passes through the air filter situated in the ceiling of the bus and continues to the evaporator where there is cooled. The cooled air comes back to the cabin of the passengers' part of the bus via special vents. The movement of the air is provided by four pairs of fans situated near to the evaporator.

The surfaces of inlet and outlet side of all filters were swabbed by dry polyester swabs and directly inoculated in blood agar and Mueller Hinton agar plates. After 24 h cultivation, the colonies were counted thoroughly, and the cultivation was led to gain pure cultures of particular bacteria. At the very beginning the pure colonies were identified by immerse microscopy. Especially *Bacillus cereus*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus flexus*, *Bacillus thuringiensis* and *Bacillus pumilus* were identified. The remainder of bacteria was identified by MALDI-TOF mass spectrometry. The Table 19 represents the dimensions of all investigated filters.

The Tables 26-36 show the results of bacteria identification and quantification id CFU/cm^2.

Table 19 The dimensions of the filters

Number of the filter	width [cm]	length [cm]	area [cm^2]
1	47	158,5	7449,5
2	47	159	7473
3	47	158,5	7449,5
4	47	158,5	7449,5
5	31	77	2387

Table 20 Number of colonies on inlet and outlet surfaces of filter 1

	Number of colonies INLET	Number of colonies OUTLET
Bacillus pumilus	2	0
Bacillus subtilis	5	0
Bacillus licheniformis	2	0
Bacillus cereus	5	0
Staphylococcus epidermidis	1	1

Table 21 Quantification of bacteria in the filter 1 represented as CFU/cm^2

	inlet [CFU/cm^2]	outlet [CFU/cm^2]
Bacillus pumilus	0,00027	0
Bacillus subtilis	0,00067	0
Bacillus licheniformis	0,00027	0
Bacillus cereus	0,0004	0
Staphylococcus epidermidis	0,00013	0,00013

Table 22 Number of colonies on inlet and outlet surfaces of filter 2

	Number of colonies INLET	Number of colonies OUTLET
Bacillus flexus	2	1
Bacillus subtilis	3	1
Staphylococcus warneri	0	1
Bacillus megaterium	1	0

Bacillus licheniformis	1	0

Table 23 Quantification of bacteria in the filter 2 represented as CFU/cm^2

	inlet [CFU/cm^2]	outlet [CFU/cm^2]
Bacillus flexus	0,00027	0,00013
Bacillus subtilis	0,0004	0,00013
Staphylococcus warneri	0	0,00013
Bacillus megaterium	0,00013	0
Bacillus licheniformis	0,00013	0

Table 24 Number of colonies on inlet and outlet surfaces of filter 3

	Number of colonies INLET	Number of colonies OUTLET
Peribacillus simplex	1	1
Bacillus subtilis	5	1
Priestia megaterium	1	1
Paenibacillus woosongensis	1	1
Brevibacillus borstelensis	1	0
Bacillus cereus	3	1
Bacillus licheniformis	2	1
Bacillus pumilus	1	1
Peribacillus muralis	1	0
Priestia endophytica	1	0
Alkalihalobacillus clausii	1	0
Bacillus flexus	0	1
Paenibacillus tylopili	0	1
Micrococcus luteus	0	1
Neobacillus niacini	0	1
Lysinibacillus halotolerans	0	1
Sporosarcina newyorkensis	0	1
Burkholderia glumae	0	1
Paraburkholderia xenovorans	0	1

Table 25 Quantification of bacteria in the filter 3 represented as CFU/cm^2

	inlet [CFU/cm²]	outlet [CFU/cm²]
Peribacillus simplex	0,00013	0,00013
Bacillus subtilis	0,00067	0,00013
Priestia megaterium	0,00013	0,00013
Paenibacillus woosongensis	0,00013	0,00013
Brevibacillus borstelensis	0,00013	0
Bacillus cereus	0,0004	0,00013
Bacillus licheniformis	0,00027	0,00013
Bacillus pumilus	0,00013	0,00013
Peribacillus muralis	0,00013	0
Priestia endophytica	0,00013	0
Alkalihalobacillus clausii	0,00013	0
Bacillus flexus	0	0,00013
Paenibacillus tylopili	0	0,00013
Micrococcus luteus	0	0,00013
Neobacillus niacini	0	0,00013
Lysinibacillus halotolerans	0	0,00013
Sporosarcina newyorkensis	0	0,00013
Burkholderia glumae	0	0,00013
Paraburkholderia xenovorans	0	0,00013

Table 26 Quantification of identified bacteria in the filter 3 represented in CFU/ml

	inlet [CFU/ml]	outlet [CFU/ml]
Bacillus flexus	2	1,5
Alkalihalobacillus clausii	0	0,5
Bacillus pumilus	0,5	0
Burkholderia glumae	0	0,5
Bacillus subtilis	4	1
Bacillus licheniformis	1,5	0,5
Bacillus cereus	1	0

Table 27 Number of colonies on inlet and outlet surfaces of filter 4

	Number of colonies INLET	Number of colonies OUTLET

Bacillus licheniformis	5	4
Bacillus cereus	4	3
Paenibacillus glucanolyticus	1	0
Staphylococcus warneri	1	1
Staphylococcus epidermidis	1	1
Micrococcus luteus	1	0
Brevibacillus borstelensis	4	3
Gracilibacillus dipsosauri	0	1
Bacillus subtilis	15	12
Peribacillus simplex	1	1
Bacillus megaterium	5	4
Bacillus thuringiensis	0	1

Table 28 Quantification of bacteria in the filter 4 represented as CFU/cm^2

	inlet [CFU/cm^2]	outlet [CFU/cm^2]
Bacillus licheniformis	0,00067	0,00054
Bacillus cereus	0,00054	0,0004
Paenibacillus glucanolyticus	0,00013	0
Staphylococcus warneri	0,00013	0,00013
Staphylococcus epidermidis	0,00013	0,00013
Micrococcus luteus	0,00013	0
Brevibacillus borstelensis	0,00054	0,0004
Gracilibacillus dipsosauri	0	0,00013
Bacillus subtilis	0,002	0,0016
Peribacillus simplex	0,00013	0,00013
Bacillus megaterium	0,00067	0,00054
Bacillus thuringiensis	0	0,00013

Table 29 Number of colonies on inlet and outlet surfaces of filter 5

	Number of colonies INLET	Number of colonies

		OUTLET
Bacillus subtilis	10	3
Bacillud licheniformis	5	3
Bacillus cereus	3	1
Bacillus flexus	2	2
Bacillus pumilus	1	1
Peribacillus muralis	1	0
Burkholderia ambifaria	1	0
Cytobacillus ocenisediminis	0	1
Cytobacillus horneckiae	0	1
Brevibacillus borstelensis	1	1
Aspergilus niger	3	0

Table 30 Quantification of bacteria in the filter 5 represented as CFU/cm^2

	inlet [CFU/cm^2]	outlet [CFU/cm^2]
Bacillus subtilis	0,0042	0,0013
Bacillud licheniformis	0,0021	0,0013
Bacillus cereus	0,0013	0,00042
Bacillus flexus	0,00084	0,00084
Bacillus pumilus	0,00042	0,00042
Peribacillus muralis	0,00042	0
Burkholderia ambifaria	0,00042	0
Cytobacillus ocenisediminis	0	0,00042
Cytobacillus horneckiae	0	0,00042
Brevibacillus borstelensis	0,00042	0,00042
Aspergilus niger	0,0013	0

Table 31 Quantification of selected bacteria in CFU/ml

	Inlet (CFU/ml)	Outlet (CFU/ml)	Efficiency (%)
Bacillus flexus	2	1,5	25
Alkalihalobacillus clausii	0	0,5	0
Bacillus pumilus	0,5	0	100
Burkholderia glumae	0	0,5	0
Bacillus subtilis	4	1	75
Bacillus licheniformis	1,5	0,5	67,7

Bacillus cereus	1	0	100

The Table 31 shows the results of quantification of bacteria in CFU/ml.

As we can see, the contamination of bus air filters is low and mainly, the environmental bacteria were detected. The contamination of outlet surface suggests that the bacteria penetrate the filter. The poor filtration effect can be seen also in the case of *St. epidermidis* and *St. warneri*, which are potential pathogens and were detected on both surfaces of the filter.

4.4. Multiplex PCR

To assess microbial contamination via nucleic acids assay, the FilmArray® (Biomerieux) detection system and QIAStat DX® Analyzer 1.0 were used. The systems are certified for use in clinical microbiology.

4.4.1. FilmArray®

All reagents required for nucleic acids detection are provided in closed reagent pouch. The main purpose and advantage of this pouch is diminishing the opportunity of procedure contamination. After hydration and sample loading, the pouch is placed to Film Array Instrument to perform a run.

Two detection sets were used. The Respiratory Panel having 20 targets is mainly focused on respiratory viruses (the Respiratory panel kit is presented in the Figure 6). The bacterial targets are in minority. On the other hand, the Pneumonia Panel with 27 targets points out bacterial pathogens causing the infections of lower respiratory tract. In addition, 7 antimicrobial resistance genes could be detected.

The Respiratory panel provides qualitative detection of infectious agent's nucleic acids. The results say if the pathogen was or did not be detected, but do not give any information of viral or bacterial load.

In Pneumonia panel, all targets are evaluated qualitatively, moreover the bacterial nucleic acid is reported semi-quantitatively with bins representing approximately 10^4, 10^5, 10^6, or $\geq 10^7$ genomic copies of bacterial nucleic acid per milliliter (copies/mL) of specimen, to aid in estimating relative abundance of nucleic acid from these common bacteria within a specimen. Despite the fact that the number of copies/ml does not correlate with CFU/ml, it can give good information about microorganism abundance in the specimen. For bacteria, negative assays (no measurable amplification or value less than 10^3.5 copies/mL) are reported as Not Detected. Positive assays are reported as Detected and a bin

result is assigned based on the assay value. Each bin is defined by discrete upper and lower limits spanning a 1-log range of values. The method is fully automated excluding the preparation of the sample and sample loading. The workflow of both the panels is visible in the table below. The sampling and kit manipulation were performed in laminar-flow class II biosafety cabinet. The surfaces and the loading station were treated with Termi-DNA-tor spray (Dynex) to avoid any contamination.

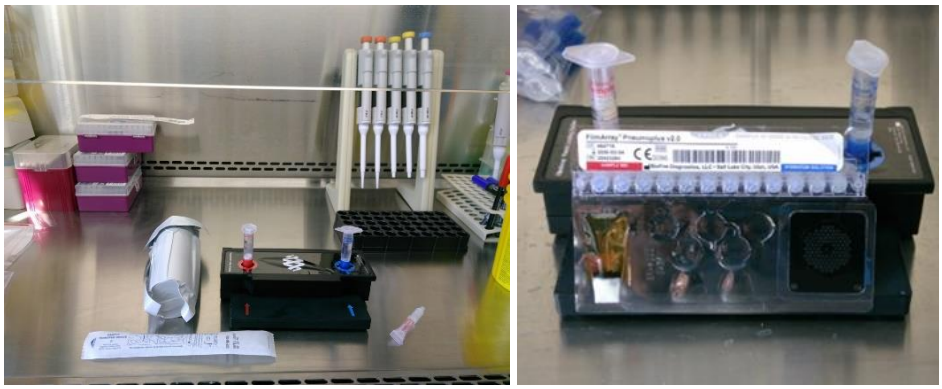

Figure 6 The FilmArray kit in the laminar-flow class II biosafety cabinet, the assembled loading station with reagent pouch, hydration injection vial (blue) and sample injection vial(red) (Daniela Obitková 2019)

4.4.2. QIAStat DX® Analyzer 1.0

QIAStat DX® Analyzer 1.0 is primarily designed for multiple pathogen search. The multiple pathogen search was beneficial for our study where the main aim was to cover as much as possible especially viral pathogens.

The dry swabs of inlet and outlet surfaces of the air filters rinsed to the tube with 1 ml of sterile saline solution. Then the dry swabs of inlet and outlet surfaces oof the air filters rinsed to the tube with 1 ml of sterile saline solution. Sample of 200 µl was transferred to the QIAStat DX® Analyzer 1.0 cartridge inlet chamber (Respiratory panel). The following Figure 7 depicts the Respiratory panel cartridge.

Figure 7 The Respiratory panel cartridge.

The internal controls were finished and whole PCR test was completed in accordance with the producer's protocols. The screenshot of the multiplex PCR run with the curve of real-time PCR of the internal control is shown in the Figure 8.

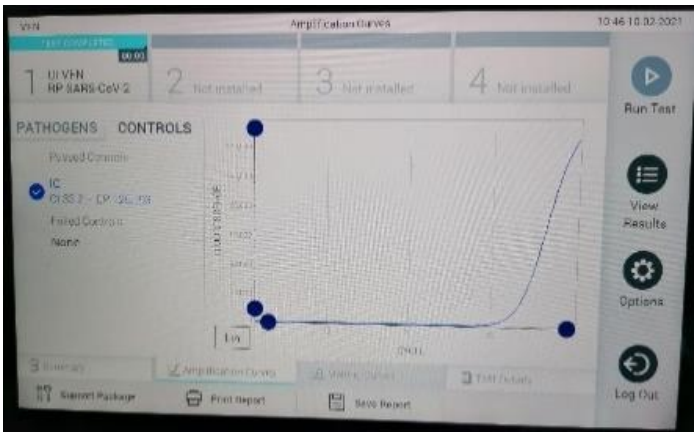

Figure 8 The PCR curve of internal control of QIAStat DX® Respiratory SARS CoV-2 Panel (the x axis represents time, the y axis summarizes the number of PCR cycles).

4.4.3. Aircraft Air Filter

Dry swabs of the inlet and outlet surfaces were performed in the same way as in the bacteriologic study. The swabs were rinsed in the sample buffer of the FilmArray® Respiratory panel and 200 μl applied to the Respiratory panel cartridge. We performed 12 runs of FilmArray® Respiratory panel – 6 inlet surface, 6 outlet surface.

Providing dry swabs and FilmArray® tests from both inlet and outlet surfaces no pathogens were detected within the Respiratory panel. Pneumonia panel was not applied.

4.4.4. Automotive Air Filters

Dry swabs of the inlet and outlet surfaces were performed. The swabs were rinsed in the sterile saline solution 1 ml, 200 μl of this sample solution was applied on the Respiratory panel cartridge, 12 runs of the Respiratory panel of QIAStat DX® Analyzer 1.0

On the surfaces of the automotive air filters no human pathogens were detected.

4.4.5. Household Air Purifier – HEPA Filter

The household air purifier and HEPA filter specification is as follows. The

dimensions of the HEPA filter are 32.5 x 15.6 x 2.5 cm. The dimensions of the whole air purifier are 39.6 cm (length), 21.7 cm (width) and 50.2 cm (height).

The investigated HEPA filter was removed from household air purifier at the end of its lifetime (6 months). Both the inlet and outlet surfaces underwent the research. Moreover, the outlet sponge filter covering the outlet side of the filter was tested. The assay was provided with FilmArray® Respiratory panel only. The HEPA filter consists of pleated filtering medium and is placed in a paper frame, covered by a porous sponge on the inlet surface as visible in the Figure 9.

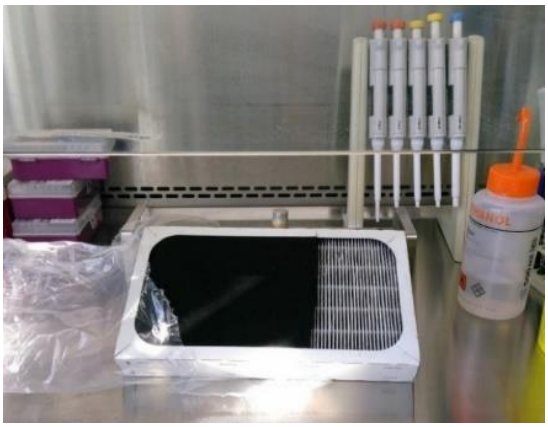

Figure 9 The HEPA filter removed from household air condition (Daniela Obitková 2019)

Dry swabs of the inlet and outlet surfaces were rinsed in the sample buffer of the FilmArray® Respiratory panel and then 200 μl of the sample buffer was applied to the Respiratory panel cartridge. 6 runs of FilmArray® Respiratory panel were performed – 2 runs for inlet surface, 2 runs for outlet surface, 2 runs for sponge filter. The results are summarized in the Table 32.

The household was inhabited by two adult people without any symptoms of respiratory disease during the period of HEPA filter change.

Table 32 Pathogens detected in HEPA filter removed from household air conditioner.

Surface	Result
inlet	NO detected pathogens
outlet	rhinovirus, enterovirus
sponge filter, outlet side of HEPA filter	coronavirus 229 E

4.4.6. Household Air Purifier – Experiment 1

A specimen of patented nanotextile material was inserted to the household air purifier to the place intended for HEPA filter. The leaks were tightened thoroughly. The nanotextile served in the air conditioner for 5 days, 24 hours a day. FilmArray® Pulmonary panel was used for pathogenic organism search. Due

to low thickness of the nanomaterial, only the inlet surface was swabbed.

Damp flocked swabs – moistened by sample buffer of the Pulmonary panel were taken. The swabs were rinsed in the sample buffer of the Pulmonary panel and 300 μl applied to the Pulmonary panel cartridge. Then 5 runs of FilmArray® Pulmonary panel were performed. The results are summarized in the Table 33.

Table 33 Pathogens detected on the nanotextile filter serving in household air purifier

Pathogens detected	Number of copies/ml
Acinetobacter calcoaceticus-baumannii complex	104
Escherichia coli	104
Serratia marcescens	105
Staphylococcus aureus	104
Coronavirus	–

Antimicrobial resistance genes were detected – CTX-M (extended spectrum β-lactamase gen), OXA-48-like (carbapenem resistance gen) as visible in the Table 34.

Table 34 Antimicrobial Resistance (AMR) Genes and Applicable Organisms

Gene	Microorganism
CTX-M	*Acinetobacter calcoaceticus* - baumannii complex
	Escherichia coli
	Serratia marcescens
OXA-48-like	*Escherichia coli*
	Serratia marcescens

4.4.7. Household Air Purifier – Experiment 2

The experiment arrangement is as follows. The household air purifier used as a model device in this work consisted of an inlet section where a fan driven by an electric motor draws air into the device. The air then leaves the device through a HEPA filter. The air filter is covered by a paper frame and placed in the plastic cell of the air conditioner. The plastic cell provided sufficient space to place a single monolayer behind the exit face of the HEPA filter as the last filter media. The size of the nanotextile was proportional to the size of the HEPA filter to reduce leakage of filtered air. The experimental setup is the following: air enters the inlet surface of the HEPA filter and continues into the filter layers. The air then exits the HEPA filter and passes through a monolayer of nanofibers. The air then exits the

unit. The experimental setup is depicted in the Figure 10.

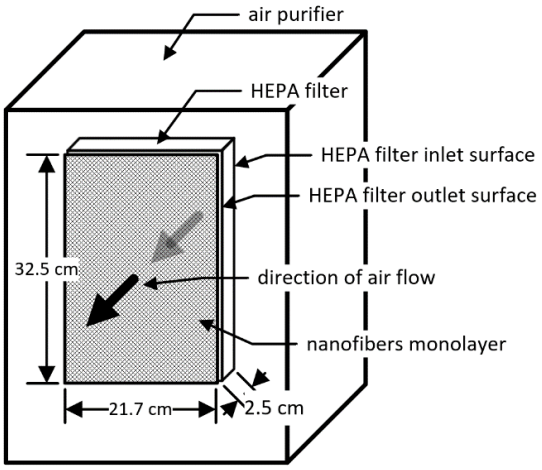

Figure 10 The household purifier experimental setup.

In this configuration, the air purifier was used for 100 h. The monolayer of nanotextile was replaced three times to prevent clogging of the nanotextile pores and degradation of the experiment. At the very end, we obtained one HEPA filter and three individual nanotextile sheets of rectangular shape and the same size as the HEPA filter. Then a sample was taken from the surface of the HEPA filter and the nanotextile monolayer. Each surface – HEPA inlet, HEPA outlet, nanotextiles 1 to 3 – was wiped with dry polyester swabs. The swabs were rinsed in 1 mL of sterile saline solution, yielding 5 samples.

We then used RT PCR method. The QIAStat DX® Analyzer 1.0 with the Respiratory SARS CoV-2 Panel (Genetica Ltd, CZ) are designed primarily for clinical use. In our experiment, we decided to use this instrument because of its simple use and wide range of target viral pathogens. The cartridges produced for respiratory panel provide two possible ways of sample applications. The whole swab or liquid sample can be inserted to the cartridge. For our experiment, we chose to apply the liquid sample exactly according to the manufacturer's instructions. Each of our five samples was applied to one cartridge, so that the QIAStat DX® Analyzer 1.0 automated cycler performed 5 separate cycles. The sixth sample was then obtained by swabbing the throat of a member of the experimental household who provided it voluntarily (volunteer in Table 35). This sample had its own cartridge and its own analysis run. The detection results are published in a qualitative manner. The measurement report contains the information – detected/not detected, the results are published in a qualitative way only. Quantification of the viral load is not available.

The RT PCR procedure revealed that the inlet surface of the HEPA filter

contained an adenovirus. It did not penetrate the filter, so the outlet surface did not contain any virus included in the portfolio of the Respiratory panel. Coronavirus 229E was not detected on either the HEPA filter inlet surface or the HEPA filter outlet surface. It was only detected on the monolayer of the nanotextile. It was detected on all three experimental parts of the nanotextile. Coronavirus 229E was detected in the throat of a volunteer from the experimental household. The RT PCR results are summarized in the following Table 35.

Table 35 Results of virus detection

Type of the filter	Detected pathogen
HEPA FILTER inlet	Adenovirus
HEPA filter outlet	NONE
nanotextile 1	coronavirus 229E
nanotextile 2	coronavirus 229E
nanotextile 3	coronavirus 229E
volunteer	coronavirus 229E

5. Conclusion

As the results suggest, the work provides good evidence of microbial contamination of air filters. In addition, the presence of pathogens proved on the outlet side of the aircraft air filter can be the evidence of impaired efficiency of filter for bacteria. The pathogens detected can recirculate in the cabin air and threaten the passenger and crew members, especially *Staphylococcus aureus* (1μm in diameter) or *Escherichia coli* (2-3 μm). The quantification of selected bacteria proved ten times higher bacterial contamination of the outlet side than the inlet surface of the filter. Based on this fact, we can recommend more frequent air filter change. The automotive air filters have not shown pathogenic bacteria and also no viruses were detected.

According to the experimental data, the automotive air filters show good function when considering personal cars used mainly by limited number of persons.

The bus air filters showed relatively low bacterial contamination. But the contamination of inlet and outlet surface was comparable. Also, the potential

pathogens *St. epidermis* an *St. warneri* were detected on both surfaces. The experiments suggest low filtration effect of $PM_{2,5}$ air filters. We recommend the use of finer filters for urban bus air conditioning systems. Considering the microbial size, the PM_1 filters could be the optimal choice.

In our experiment, the Coronavirus 229E repeatedly penetrated the conventional HEPA filter. The simulations with household portable air purifier, where the nanotextile was tested as a final filtration medium showed the ability of the nanomaterial to capture the coronavirus.

To sum up, the existing results are in significant accordance with the statement that current air filters are not efficient enough in pathogen removal.

To conclude, the outcomes of this dissertation include the following suggestions:

1) More frequent exchange of aircraft air filters is recommended.
2) Use of finer air filters in the urban buses air conditioning system is recommended.
3) The efficiency of conventional air filters could be enhanced by addition of nanotextile as a final air filtration medium.

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7. List of Publications Related to the Dissertation

OBITKOVÁ, D. a M. MRÁZ. Viruses in High Efficiency Air (HEPA) Filtration and Nanofiber-Based Nanomaterials Potential for Nanoparticle Filtration. *SPEKTRUM*. 2023, **23**(2), 11-17. ISSN 1804-1639. Dostupné z: https://www.fbi.vsb.cz/export/sites/fbi/cs/.content/galerie-souboru/Spektrum/Spektrum_2023_2.pdf

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8. List of Publications not Related to the Dissertation

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HEŘMAN, T., P. BOHM a D. OBITKOVÁ. Srovnání třídících systémů ve zdravotnických zařízeních. In: *Recenzovaný sborník příspěvků mezinárodní vědecké konference Mezinárodní Masarykova konference pro doktorandy a mladé vědecké pracovníky 2019*. Mezinárodní Masarykova konference pro doktorandy a mladé vědecké pracovníky 2019, Hradec Králové, 2019-12-16/2019-12-18. Hradec Králové: Magnanimitas, 2019. s. 296-304. sv. X.. ISBN 978-80-87952-31-3.

9. Citations

There are no known citations at the time of submitting the dissertation thesis.

Summary

Airborne infectious diseases transmission is an important public health issue. To evaluate the air conditioning system contribution to air borne microorganism transmission, the contamination of four kinds of air filters were investigated including the pilot experiments where the nanotextile was used to enhance the filtration capacity of conventional high efficiency particulate air (HEPA) filter.

The qualitative and quantitative bacteriological assessment and multiplex PCR assay were performed. In the air filter removed from a commercial aircraft the detection revealed *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae or Clostridium tetani.* Amongst Gram negative bacterial species *Pseudomonas aeruginosa, Morganella morgani,* and *Escherichia coli* were identified. Quantitative study of selected bacteria contaminating the filter showed 10^6 to 10^7 CFU/ml. The air filter extract surface contamination was about ten-fold higher than the inlet side of the filter. The multiplex PCR technique did not show any viral nucleic acids.

Automotive and bus air filters bacteriological study detected mostly environmentally present bacterial species as *Bacillus, Brevibacillus, Peribacillus or Burkholderia and Paenibacillus.* The automotive air filters fitting the category of porosity $PM_{2.5}$ were free of any bacterial or viral pathogens. The bus air filters belonging also the category of $PM_{2.5}$ *Staphylococcus epidermidis* and *Staphylococcus warneri* were detected as potential pathogens.

In the HEPA filter coming from the household air purifier firstly the occurrence of pathogenic viruses was examined. The multiplex PCR assays detected the Coronavirus 229 E, Rhinovirus, Enterovirus and Adenovirus repeatedly. Portable air purifier equipped with the HEPA filter served as a model device in pilot experiments with nanotextile filter medium. The main objective of the study was to determine microbial contamination on the HEPA filter and to investigate if the selected nanotextile monolayer made of polyamide 6 (PA6) nanofibers can capture potential microorganisms when installed downstream the HEPA filter as the final filtration medium. Adenovirus was detected on the inlet surface of the HEPA filter. The outlet surface of the filter contained no viruses. The nanotextile monolayer was replaced twice during 100 h of operation, so three pieces were used and all of them contained coronavirus 229 E. The study showed that the selected nanotextile is capable of capturing a virus of a small size.

Resumé

Přenos infekčních nemocí šířících se vzduchem je důležitým problémem zásadně ovlivňujícím veřejné zdraví. K posouzení, zda klimatizační systémy k mohou napomáhat šíření mikroorganismů v cirkulujícím vzduchu, byla zkoumána mikrobiální kontaminace čtyř druhů vzduchových filtrů, včetně pilotních experimentů, kde byla použita nanotextilie k posílení filtrační kapacity konvenčních vysokoúčinných částicových (HEPA) filtrů.

Bylo provedeno kvalitativní a kvantitativní bakteriologické hodnocení včetně multiplexních PCR testů. U vzduchového filtru získaného z komerčního letadla detekce odhalila *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae* a *Clostridium tetani*. Mezi Gram-negativními bakteriálními druhy byly identifikovány *Pseudomonas aeruginosa, Morganella morgani* a *Escherichia coli*. Kvantitativní studie vybraných bakterií ukázala počty kolonií od 10^6 do 10^7 CFU/ml. Kontaminace výstupního povrchu vzduchového filtru byla desetkrát vyšší než povrchu vstupního. Technika multiplexního PCR nezjistila žádné virové nukleové kyseliny.

V bakteriologické studii automobilových a autobusových vzduchových filtrů byly detekovány převážně příslušníci bakteriálních rodů běžně se vyskytujících v prostředí, jako jsou rody Bacillus, Brevibacillus, Peribacillus, Burkholderia a Paenibacillus. Automobilové vzduchové filtry spadající do kategorie poréznosti $PM_{2.5}$ byly shledány bez bakteriálních či virových patogenů. Naopak, u autobusových vzduchových filtrů, které také spadaly do kategorie $PM_{2.5}$, byly zjištěny potenciální patogeny *Staphylococcus epidermidis* a *Staphylococcus warneri*.

U HEPA filtru získaného z domácí čističky vzduchu byly detekovány Coronavirus 229 E, Rhinovirus, Enterovirus a Adenovirus. Přenosná čistička vzduchu vybavená HEPA filtrem byla použita v pilotním experimentu, kde byla zařazena nanotextile za HEPA filtr jako poslední filtrační medium. Hlavním cílem studie bylo zjistit mikrobiální kontaminaci HEPA filtru a ověřit, zda vybraná nanotextilní jednovrstvá membrána z polyamidu 6 (PA6) může účinně zachytit potenciální mikroorganismy. Na vstupní ploše HEPA filtru byl detekován Adenovirus, zatímco na výstupní ploše nebyly nalezeny viry žádné. Nanotextilní jednovrstvá membrána byla během 100 hodin provozu vyměněna dvakrát; všechny tři použité kusy obsahovaly Coronavirus 229 E. Studie ukázala, že vybraná nanotextile je schopna zachytit viry malých rozměrů.