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PERFORMANCE OF DIFFERENT TYPES OF AIR FILTERS IN INFECTIOUS AGENTS INTERCEPTION

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Declaration

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Abstract

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.

Keywords

Air filter, bacteria, microbial contamination, multiples PCR, virus

Abstrakt

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.

Klíčová slova

Bakterie, mikrobiální kontaminace, multiplexní PCR, viry, vzduchové filtry

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1. Introduction

Due to the increasing ease and affordability of traveling and mobility of people, airborne infectious diseases transmission during traveling and stay in air-conditioned spaces represents an important public health problem.

The serious danger and the population protection issue can be the possibility of transmission and spread of microorganisms via the air conditioning (AC) system. The air conditioning system is an indispensable equipment of the aircraft, vehicles of public transport, residential and office buildings. The numerous reports about many diverse pollutants present in the indoor air of different spaces were issued. The air within the cabin of the means of transport, homes or offices may contain residues of fuels, oil vapors and different particulate matter. The air conditioning system recirculates the air and the air filters collect majority of undesirable pollutants. The microorganisms especially the air borne ones contaminate the air wherever people are present. All particulate matter including microbes is sucked to the air conditioner conduit and reach the air filter. The air filters are capable of absorbing smells or capture dust and majority of the solid particles. The COVID-19 disease pandemic raised the interest in air filtration and protection of human health against highly contagious infectious diseases.

The presented study is focused on the air filters microbial contamination, analysis of bacterial and viral contaminants. The research was aimed at filtration effectiveness assessment. At first, the answer to several questions was searched. When considering the magnitude and different shapes of bacteria of the ultrafine magnitude of viruses (20-120 nm in diameter), are the air filters capable to collect them effectively? Is the effectiveness of currently used air filters sufficient to eliminate the microorganisms from filtered air? In the times of nanomaterial which demonstrate precise characteristics in air filtration, the study suggested a possible way of standard air filter efficiency enhancement.

2. Problem Formulation and Approach to Solution

From ancient times to current days, the infectious diseases and their transmission and spread represent a threat for human beings. Recently, the main documents dealing with safety and population protection stated the potential origin of pandemic or epidemic as the severe threats. The Safety strategy of the Czech Republic includes spread of highly contagious infection diseases among the current threats to our country (*Bezpečnostní strategie České republiky* 2015).

The Population Protection Conception valid to the year 2030, with prospects to the year 2050 evaluates infectious diseases as a threat and extends the problem of infectious diseases transmission to the area of terrorism – bioterrorism in particular (Vláda České republiky 2013),(*Koncepce environmentální bezpečnosti 2016-2020 s výhledem do roku 2030* 2015).

A wide variety of diverse routes of infectious diseases transmission, especially caused by air borne pathogens, come into regard. As previously reported air travel, public transport and enclosed air-conditioned spaces can significantly contribute to infectious agent spread. Both the infected person, either sick or in incubation period, and the potential bioterrorism attack should be taken into consideration.

Each air conditioning system contains an efficient air filter that should capture particles dispersed in the air. Regarding the pathogenic organisms, some of bacteria and majority of viruses are significantly small and can penetrate the air filters.

Assuming all these statements a wide variety of questions emerged:

- What microorganisms contaminate the air filters?
- Which human pathogens could be present in this contamination?
- Can the human pathogens penetrate the filter? If so, is there any device to arrest the infectious agents?

According to these questions several premises were stated:

1) The air filters are contaminated with microorganisms pathogenic for humans.
2) The microorganisms can penetrate conventional air filters.
3) The nanomaterial with lower porosity than the commonly used air filters suitable for air filtration can capture the human pathogens and have good potential to enhance filtration capacity of conventional air filters.

The microbiological study of air filters will be provided employing the

methods using standardized detection techniques for pathogenic microorganisms in routine diagnostic procedures.

- Cultivation of viable bacteria
- Identification of bacterial species – biochemical tests, microscopic techniques, proteomic study (MALDI TOF)
- Molecular biology techniques identifying viruses and bacteria – real time polymerase chain reaction (RT-PCR)
- Identification of a model device to test air filtration by conventional air filter enhanced by addition of defined sample of nanomaterial to evaluate the nanomaterial filtration potential.

3. 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.

4. 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.

4.1. 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

In the front part of the airplane the avionics ventilation system is situated. This system provides cooling of the air circulating within the avionics system. The heat or hot air produced by the instruments is cooled via circulation close to the skin of the aircraft to prevent instrument overheating.

In general, the aircraft air conditioning system is arranged as Environment control system. It means that the air conducted to the cabin comprises the air from outside environment mixed with the recirculated air. The outside air is sucked by

the engines and collected via efficient compressors. The air passing through the engines is very hot, 200-300°C, and is led to the pressure-and-air conditioning kit (PACK). This device provides cooling and proper pressurization of the air. The Airbus aircraft have two PACKS. Some hot air avoids the PACKs and is used to direct temperature control in the fuselage compartment. The air leaving the PACK reaches the mixer unit where both the outside and recirculated air are mixed and supplied to the cabin.

In terms of air conditioning description, the aircraft fuselage is divided into three compartments – flight deck used by the crew members, forward cabin and aft cabin. The picture below shows the cabin airflow arrangement. The overhead ventilation unit leads the cleaned air to the cabin via gasper, the air is then extracted via cabin exhaust air outlet situated near the floor. About one half of this air is exhausted from the airplane through an outflow valve in the underside. Remaining one half of the cabin air is intended for recirculation and flows to the filters visible in the bottom, right and left side of the picture. The air flow arrangement produces laminar flow across the airplane. The front-to-back flow (longitudinal) is in minority. This air circulation pattern divides the air flow into sections within the cabin, thereby limiting the spread of airborne particles throughout the passenger cabin.

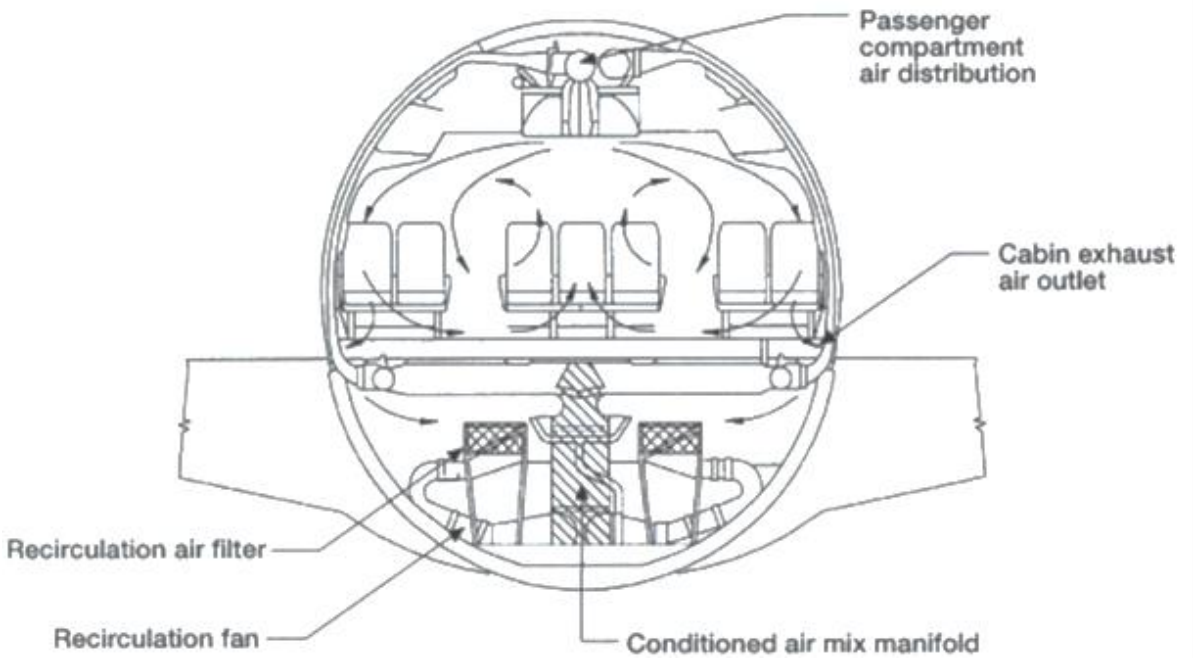

Figure 1 The air flow inside the passengers' cabin (Díaz, 2011)

Despite the diversity of airplanes, the arrangement and Environmental Control System is very similar in majority of types. The fresh air (bleed air) provided by engines represents on average 50% and the recirculated air 50% of the cabin air. The usual air velocity ranges between 0.05 m/s and 0.3 m/s. The velocity above 0.3 m/s can cause draft sensation on the neck. Ventilation capacity varies substantially, dependent on the aircraft type but typically averages at 4.7 L/s (National Research Council 2002). The cabin air is maintained dry. The bleed air, essentially dry is mixed with recirculated air containing the vapors produced by passengers and crew. A sedentary passenger produces 0.7 g/min, the crew member 2 g/min of vapor. The recirculated air is dried and the overall humidity is maintained on low level. According to the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE) standard, the humidity maximum refers to 20% of relative humidity. The cabin pressurization is usually maintained at the level of 2400 meters above the sea level. This value refers to the limit (the cabin altitude may not exceed 8000 ft) issued by Federal Aviation Administration of the United States of America (FAA) (Hocking 2002).

4.2. Air Contaminants

4.2.1. Air Contaminants of the Aircraft Cabin

When considering sources of the air for cabin ventilation, the air can achieve specific contaminants from generally several sources. At first, the chemical pollutants can come from the engines and air conditioning mechanical components. Microbial contamination can arise from outside air or from human beings travelling by the aircraft.

4.2.1.1. Chemical Contaminants

As mentioned before, the bleed air drawn from jet engines and supplied to the cabin via the mixing unit can contain various contaminants of different types including ketones, volatile organic compounds, fumes from oil leaks or smog from engine exhaust. This contamination can occur during normal operation or incidentally. In normal operation carbon monoxide CO, carbon dioxide CO2 or organic phosphates were considered as main cabin air contaminants. Flying in high altitude levels, ozone contamination may occur.

Pyrolysis of commercial jet oils could release a range of substances into cabin

air during a seal failure including cresyl- and the more volatile butyl-phosphate esters (Day 2010). During normal operation the levels of chemical contaminants does not exceed the toxicological limits and the quality of the cabin air is not influenced ("Air Travel and Health: an Update Report with Evidence" 2008), (Day 2010).

4.2.1.2. 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.

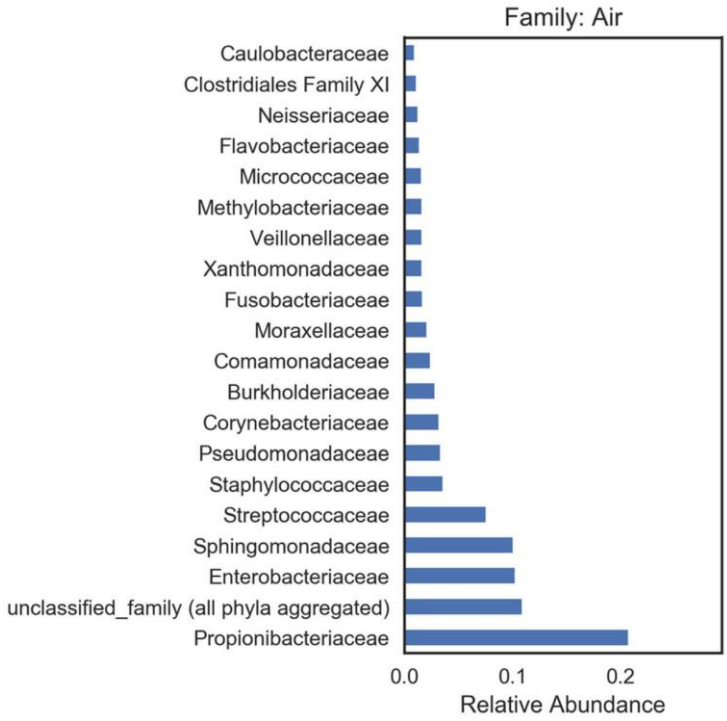

Figure 2 Bacterial families in the cabin air of the aircraft (Weiss et al. 2018)

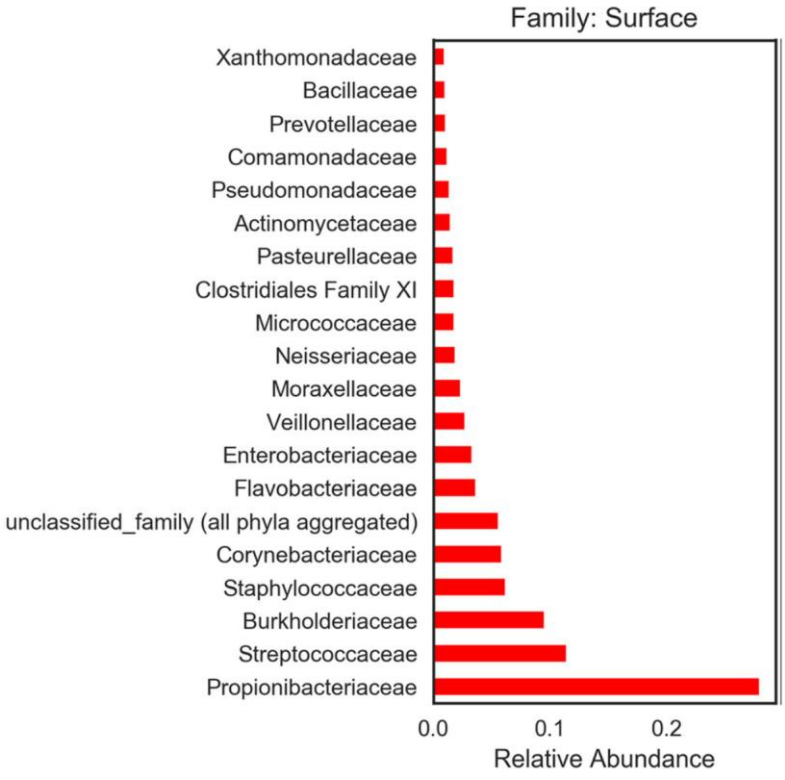

Figure 3 Bacterial families on touch surfaces of the aircraft (Weiss et al. 2018)

Studies finding the bacteria in aircraft air samples using both cultivation of viable bacteria and molecular biology techniques including quantitative PCR revealed many different bacteria present onboard. In the U.S, domestic flights were used, assessing the microbes in economy class cabin air. The results suggest the circulation of microorganisms within the cabin. The authors shoved the bacterial burden in the cabin air ranging from below detection limits to $4.1x10^6$ cells/m3. Among all bacteria detected, *Neisseria meningitidis* or *Streptococcus mitis* represent pathogenic and opportunistic pathogenic inhabitants of the human respiratory tract and oral cavity (La Duc, Venkateswaran, and Stuecker 2007). In this study the research team also showed an interesting phenomenon of rapid increase in bacterial counts at the very beginning of the flight and decreasing of viability of bacterial species during the flight, when most viable bacteria were detected during the boarding time and midflight with sharp decrease of microbial burden and viability during initiating of descent and landing. This phenomenon could be explained by boarding the passengers containing the wide variety of microbes and their elimination during the flight duration.

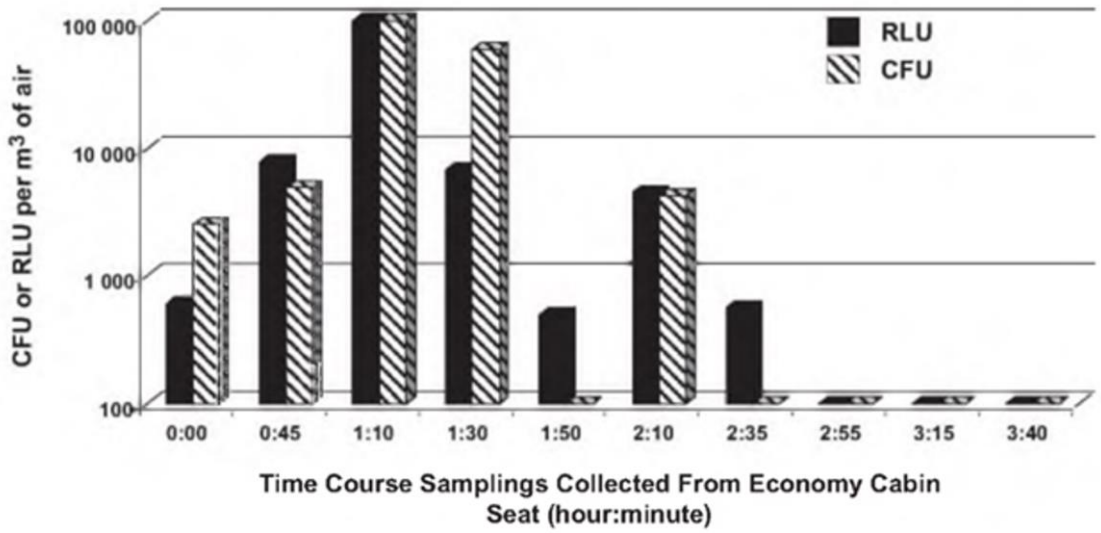

Figure 4 Changes in bacterial burden of cabin air collected at seat height of commercial airline from the western to the midwestern part of the US. Air samples were collected at 20–25 min intervals over the 3 h 40 min flight duration. RLU, relative luminescence units; CFU, colony forming units (La Duc, Venkateswaran, and Stuecker 2007).

The authors of the study also bring the concept of biofilm formation. As proved within the study, the bacterial count contained in the gasper air,

previously thought as the cleanest air source, rose instantly after passengers entered the cabin. The narrow space of the gasper was identified as suitable for biofilm origin. From this biofilm, the bacteria could be relieved immediately to the cabin air.

Investigating the microbial burden of cabin air, other research team assessed also the U.S. domestic and international airlines, using the samples from business class. The Boeing aircraft 747, 757 or 777 models operated on the chosen airlines. Using both the cultivation and molecular biology techniques, a wide variety of different mostly non-pathogenic bacteria were detected. Only several human pathogens represented by *Salmonella typhi*, or *Staphylococcus aureus* were detected. *Staphylococcus haemolyticus* represents the opportunistic pathogen retrieved in the samples (Osman et al. 2008). Both mentioned studies also employing the molecular biology techniques (quantitative PCR) suggest that bacteria can penetrate the HEPA filters and recirculate in cabin air.

The airborne microorganisms usually are transported in the air within the aerosolized droplets. When the air is circulated, the bacteria drift in the air. When the air circulation is stopped, the bioaerosols start to sediment. The size of the air contaminating particles influences the velocity of sedimentation significantly. When considering the particles from 1 μm to 40 μm, the smaller fall out slower than the larger (Pasquarella, Pitzurra, and Savino 2000). This could be important onboard – the small viral particle may stay in the air much longer than bacteria falling out faster. The Table 1 shows the overview of disease-associated bacteria onboard.

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

Acinetobacter calcoaceticus
Acinetobacter junii
Gemella haemolysans
Staphylococcus haemolyticus
Streptococcus mitis

Several studies dealing with presence of viruses onboard and air filter contamination were carried out. The viral particles are much smaller than bacteria and deeply below the diameter of 300 nm what is the particle size for testing the

effectiveness of HEPA filters. In the USA, the 48 air filters from commercial aircraft operating on both domestic and international airlines were investigated to assess the presence of respiratory viruses. The glass-fiber HEPA filters serving from 500 to 15000 flying hours were disassembled and tested for respiratory viruses. Multiplex PCR method (ResPlex II, Quiagen) was employed to provide the test. The results showed several viruses present in the filters. Three filters were detected positive containing rhinovirus, influenza A and Influenza B viruses. All remaining 45 did not contained detectable viruses by chosen technique. Interestingly, a time period from 10 to 22 days passed between filter removal and ResPlex assay, which may suggest long-term viral air filter contamination. But the viral nucleic acid detection by PCR techniques may not necessarily mean the presence of active virus in infectious amount. The viral nucleic acid assay detects both alive and inactivated forms of viruses. Nevertheless, airborne rhinovirus has been detected in office buildings, influenza virus was present in the air of hospital waiting room (Korves et al. 2011). Yang et al. focused on the influenza virus proved this item in indoor environment and also in the cabin of an aircraft using the qPCR technique (Yang, Elankumaran, and Marr 2011). While the qPCR method is a powerful tool for determining the presence of viral genomic material, it does not indicate whether the virus is viable or not.

To sum up, the recent studies dealing with microorganisms both in the cabin air and in the air filters proved presence of a wide variety of bacteria and several viruses. These results may suggest the real possibility of getting infected onboard.

4.2.2. 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.

Amongst frequently discussed diseases relevant for air travel, measles, tuberculosis, severe acute respiratory syndrome (SARS, a non-typical pneumonia caused by a coronavirus), influenza and common cold are usually mentioned. Several vector-borne diseases are also included. To evaluate the risk of contagious disease transmission onboard is very difficult, usually due to lack of relevant passenger information or incomplete questionnaire collection. Currently collected data suggest that the risk of disease transmission to other symptom-free

passengers within the aircraft cabin is associated with sitting within two rows of a contagious passenger for a flight time of more than 8 h.

But it is not possible to apply this rule generally. In the case of severe acute respiratory syndrome (SARS) the infected individuals sat far from an infected passenger. As the picture shows, the infected persons were distributed all over the cabin. This situation excludes the direct transmission as a single cause of SARS transmission to other passengers. The flight from Beijing to Hong-Kong carried one symptomatic person and 119 other passengers. Sixteen persons were reported to have laboratory-confirmed SARS, two persons were identified as probable SARS (Olsen et al. 2003).

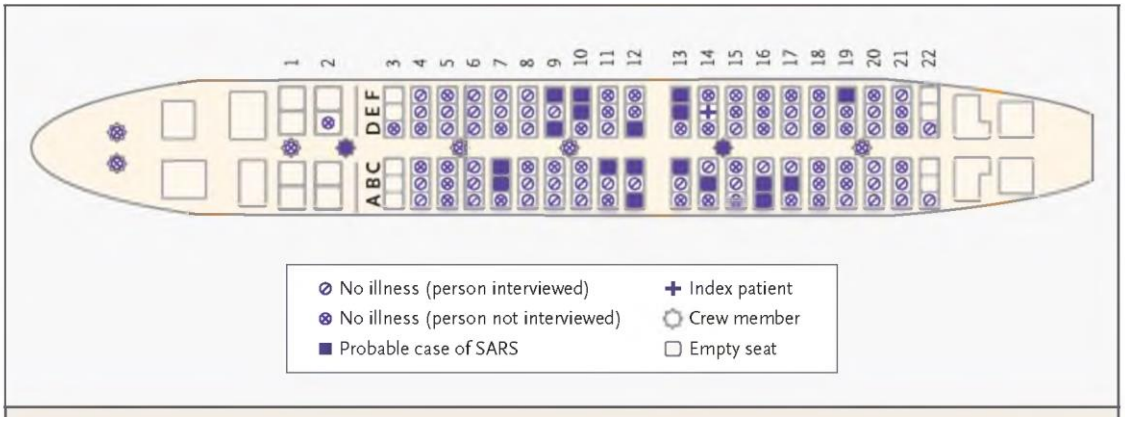

Figure 5 The flight from Beijing to Hong-Kong showing the distribution of infected persons onboard (Olsen et al. 2003)

When considering viral diseases, the influenza transmission onboard was reported as well. Majority of passengers that were infected by the influenza virus were sitting in the distance of up to two rows from the infected person. According to World Health Organization guidance, the passengers within the two seat rows are traced to identify the primary contacts and possible infected persons. In case of influenza A infection, the secondary contacts infected in longer distances than two rows were reported (Leitmeyer and Adlhoch 2016).

The cases of common cold are the most difficult to evaluate as onboard infection. The common cold or upper respiratory tract infections are the non-homogenous group of ubiquitous diseases which may not be assessed reliably as onboard transmission.

Inflight measles transmission was also reported. Until now several cases

worldwide were described. Usually, the infected persons were in close proximity to the index passenger, or in one case the crew member was the source of the virus onboard.

Moving to bacterial diseases, the meningococcal meningitis should be taken into consideration. A case of meningococcal disease associated with air travel is defined as the development of the illness within 14 days of travel on a flight lasting at least 8 h, including ground delay, take-off and landing. The passengers flying with meningitis symptoms were reported but no onboard transmission have been described yet.

Tuberculosis represents dangerous and contagious disease which onboard transmission was studied thoroughly. Nowadays, the inflight transmission was stopped by carefully prepared WHO Guidance, but several cases mentioned in history of air travel are known (Mangili and Gendreau 2005).

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

4.2.3. Biofilms

As mentioned above, the aircraft air conditioning conduit may give rise to biofilms. A layer of microorganisms covering the surface of air handling system of the air conditioning device could represent a permanent and resistant source of microbes for the cabin air.

Biofilms are matrix-associated microbial accretions that adhere to the biological or nonbiological surfaces. The habitats which tend to biofilm formation are mainly surfaces exposed to flowing water. But the biofilm formation can occur

in diverse areas ranging from plant roots to human lungs or medical devices implanted to human body. The planktonic lifestyle is very different from attached lifestyle. This attached way of existence strongly influences majority of biochemical, genetic and physiological responses of microorganisms in biofilm. The biofilm development has several typical features including:

- The microorganisms have to have specific device (e.g. flagella) to reach and adhere to the surface
- The microorganisms express specific genes to obtain particular products enabling their division and proliferation producing a monolayer
- Specific gene products controlling microcolonies formation
- During the biofilm lifetime, the network of three-dimensional towers is formed
- The life of certain biofilm is dynamic and individual cells can be released to the environment (Madsen, 2015)

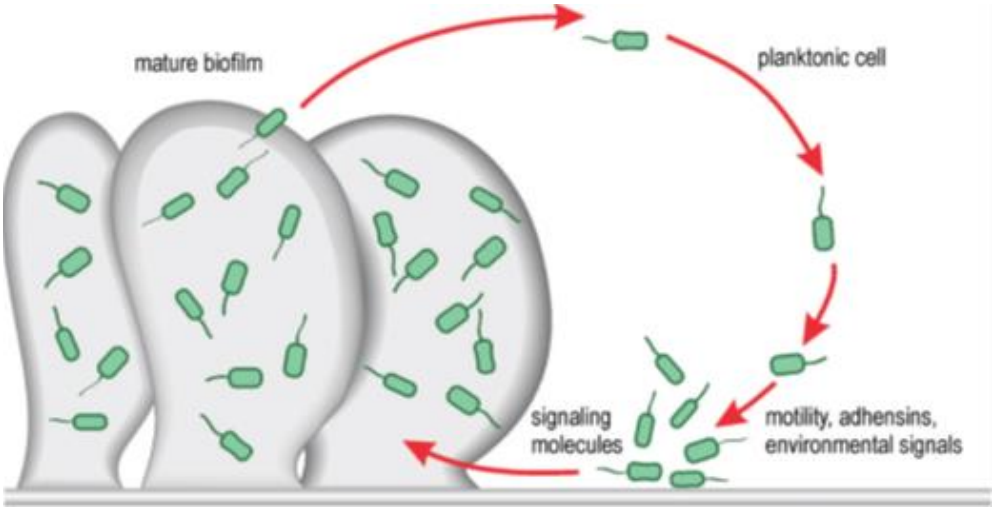

Figure 6 Biofilm formation (Maric and Vranes 2007)

A particular way of biofilm formation is species-specific. To live in biofilm can provide several reasonable advantages to the microorganisms. The biofilm layer is usually covered by a layer of organic compounds to give significant protection for the biofilm inhabitants. This may be the reason of biofilm resistance. To get rid of the biofilm especially in narrow tubes is nearly impossible. In humans or in animals, the microorganisms can gain antibiotic resistance via the plasmid transfer more easily in the biofilm. In this case, the changes of pathogenicity were also observed. The close proximity of cells facilitates mutualistic or synergistic associations as well.

Adhesion of microorganism to abiotic surface is usually nonspecific including hydrophobic interactions. The first phase of adhesion is weak and reversible and continues to permanent irreversible adhesion of microorganism. When considering the living tissues, the microorganism adhesion seems more complicated, because there the interaction between microorganism and macroorganism occurs. The bacteria have special molecules adhesins enabling the adherence and the tissue cells have specific receptors. For example, *Staphylococcus epidermidis* and *Staphylococcus aureus* produce a polysaccharide intercellular adhesin (PIA), which is associated with cell-to-cell adhesion and subsequent biofilm formation. In humans several diseases having origin in biofilms were described. The Table 3 describes the list of several biofilm-associated diseases. Some of them are connected to biofilms formed on the tissue surfaces, several diseases are associated with medical devices implanted to the human body (Percival et al. 2011).

Table 3 Clinically significant biofilm-associated diseases (Percival et al. 2011)

Disease	Typical biofilm organism
Dental caries	*Streptococcus spp.*
Periodontitis	*Fusobacterium nucleatum, Porphyromonas gingivalis, Bacteroides forsythus, Prevotella intermedia*
Otitis media	*Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, S. aureus, S. epidermidis, P. aeruginosa*
Cystic fibrosis	*P. aeruginosa, Burkholderia cepacia*
Chronic wounds	*Staphylococci, Streptococci, Enterococci,* facultative anaerobic Gram-negative bacilli, anaerobic bacteria such as *Fusobacterium spp.* and *Peptostreptococci*
Foreign body/medical device infection Urinary catheters	*Proteus mirabilis, Morganella morganii, P. aeruginosa, Klebsiella pneumoniae, Proteus vulgaris*
Foreign body/medical device infection Native valve endocarditis (NVE)	*Streptococci, Staphylococci, Gram negative bacteria, fungi*

The biofilms of human body are under broad investigation and the knowledge of potential biofilms risk grows each day. Also, the biofilm formation in environmental habitats as in plants and plastic or metallic devices serving in flowing water are well known. With regard to aircraft and aviation, the biofilm associated corrosion was described. The microorganisms can adapt to various conditions and survive in environments hostile for other forms of living individuals.

As mentioned before, several notes about biofilm formation in the aircraft air recirculation conduit were suggested. It could be dangerous especially in the case of gasper as the last part of the air conditioning system flowing the air into close proximity of sedentary passengers. The human pathogens as Neisseria meningitidis, Pseudomonas aeruginosa, Escherichia coli or Vibrio cholerae may have the biofilm formation potential (Madsen 2015), (Kyungcheol et al. 2004). Currently the biofilms in the air conditioning systems are poorly understood and should be investigated from wide perspectives. The Table 3 summarizes biofilm-associated diseases and their causative agents.

4.3. Automotive Air Conditioning System

Automotive air conditioning is now almost universal. Mobile air conditioning systems for cars (MAC) generally use engine-driven compressors with refrigerant (Hungy, Trott, and Welch 2016). There are two main types of air conditioning systems in use in the automobile industry. The main difference between each system is the type of device that is used to lower the refrigerant pressure. Either an expansion valve or orifice tube is used. The air conditioning system has several basic parts as compressor, condenser, blowing fan, expansion valve and evaporator. The general scheme of automotive air conditioning system with expansion valve is shown in the Figure 7. The compressor draws the low-pressure refrigerant from the evaporator and compresses the refrigerant into a high-pressure refrigerant vapor, which is then sent on to the condenser. The hot high-pressure refrigerant vapor enters the tap of the condenser and forces down through the tubes of this special heat exchanger. The heat that was absorbed by the refrigerant vapor from the interior of the automobile is then released to the atmosphere. Then the vaporized refrigerant is cooled in the evaporator and sent back to the system to enter the new cycle of compression (Rațiu et al. 2018).

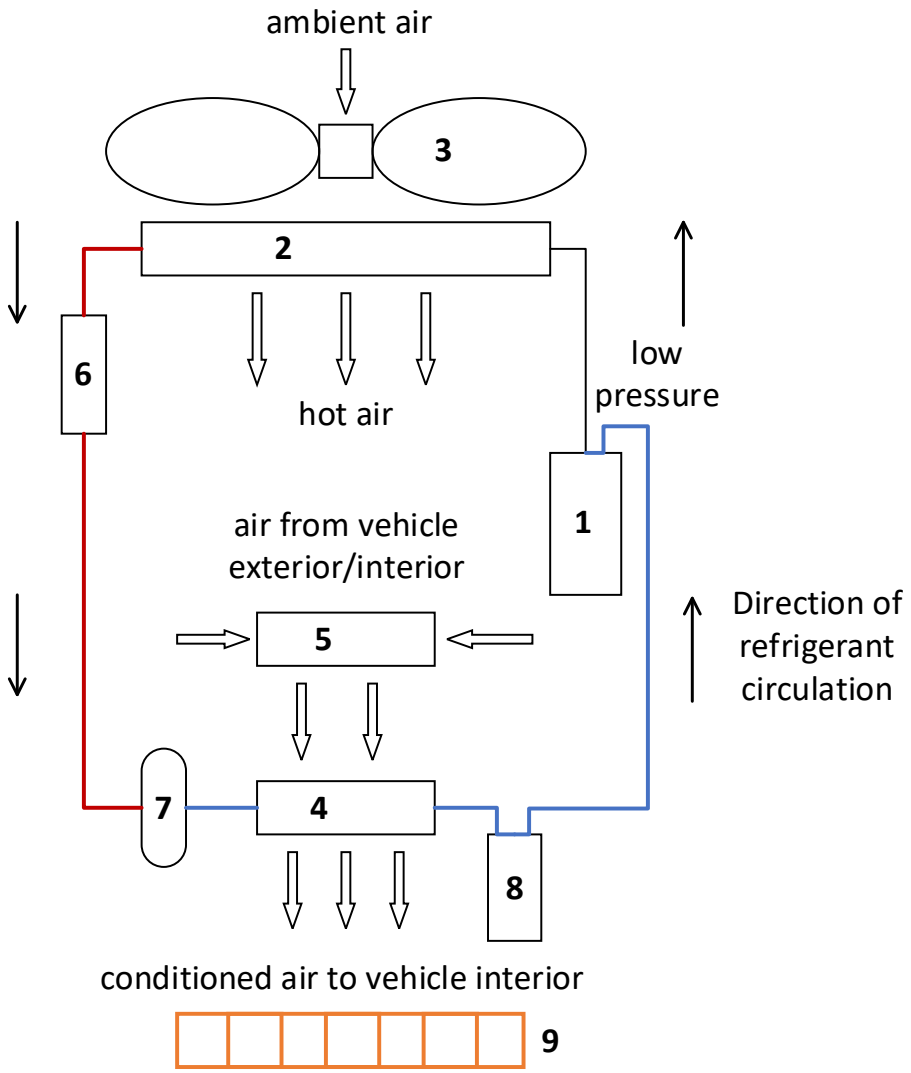

Figure 7 The general overview of automotive air conditioning system. (1 compressor, 2 condenser, 3 fan blowing the air through the condenser, 4 evaporator (cool) 5 blower – pushes the cooled air to the cabin, 6 receiver/driver where the refrigerant is collected, filtered and dried, 7 expansion valve – 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 7. 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.

4.4. Bus Air Conditioning System

The bus public transport is widespread all over the world. The buses of municipal public transport or the coaches operating on long distances transport thousands of passengers per day. Air conditioning system is an implied equipment of the buses during the warm season of the year, considering our country.

Technically, we can categorize the bus passenger's cabin, air conditioning units according to the way of drive. The independent air conditioner is driven by the electromotor. The non-independent air conditioning system is driven by the bus motor which is interconnected with the compressor of the air conditioner by the band. The non independent air conditioning system is typical for majority of big buses operating in the municipal public transport in our country. The air conditioning unit is usually placed on the outside surface of the bus roof. The unit intended for cooling consists of evaporator and ventilators driving the undesired heat to the ambient space (Figure 8). In the ceiling of the bus there is situated the air filter covered in the metallic frame (Figure 9).

Figure 8 The air conditioning unit on the rooftop of the bus (viewed from the roof of the bus), 1 the evaporator, 2 the ventilator moving the air back to the cabin of the bus, 3 the ventilator removing the undesired heat to the ambient air. (Daniela Obitková 2023)

Figure 9 The air filter of the air conditioning system in the ceiling of the bus: 1 the air filter in the metallic frame, viewed from the bus interior. (Daniela Obitková 2023)

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 the bus, the air circulation is typical. The air is sucked from the passenger section of the bus in horizontal direction and enters the front-end air filter. Then the air continues along the evaporator, where the air is cooled. The ventilators near to the evaporators run the air back to the cabin of the bus. The cooled air enters the cabin via the openings situated lateral edges of the internal surface of the ceiling of the bus cabin.

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.

The driver has separate independent air conditioning unit, usually driven by small electromotor. The air conditioning system is based on cool water aerosol.

The ground transportation by buses represents the risk of infectious diseases transmission. There are several risk factors present especially in municipal public transport. At first, the public transport buses may be crowded. Then the distances between passengers may be too short. Secondly, in the transit and coach buses the passengers stay for long time period in close air-conditioned space. In the vehicles intended for ground transportation, three routes of infectious agents are possible.

- via droplets expelled from the nose or mouth of an infected person to another person who is in close proximity
- via contact with surfaces that have been contaminated
- via airborne transmission through aerosols (Tang, Tellier, and Li 2022)

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).

4.5. Portable Air Purifiers

In current times the United States Environmental Protection Agency (EPA) says that most of humans in America spend more than 90% of the day indoors and are continuously exposed to indoor pollutants, such as fine dust ($PM_{2.5}$, PM_{10}) and airborne bacteria, viruses, molds and their spores (United States Environmental Protection Agency 2018). The European situation resembles United States situation precisely as said by European Commission(European Commission 2003). Especially the individuals suffering from respiratory, cardiovascular or eye

diseases may be sensitive to higher concentration of pollen, fine and ultrafine dust which can carry bacteria of the spores. The indoor spaces include households, offices, schools, hospitals with inpatient and outpatient departments, kindergartens and areas intended for indoor sports.

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.

As mentioned in the section about bus air conditioning systems, the air purifiers can contribute to improve the indoor air quality significantly. In the case of classrooms, the researchers investigate the influence of use of a portable air cleaner and ventilation system on air quality in the particular classroom. The ventilation system provided sufficient amount of outdoor air to provide adequate CO_2 concentrations. The results also showed 95% effectiveness of air purifiers in ultrafine and coarse dust elimination. Then, the effectiveness 82–88% in capturing particles 0.3–2 μm. So, the authors sum up that the ventilation system in combination with air purifier can maintain adequate indoor air quality of the classroom (Aldekheel et al. 2022). Another case of profitable use of HEPA filter air purifiers can be elimination of airborne fungi. It is applicable for houses, where the airborne fungi and their spores could cause allergies and for special hospital wards where reduction of airborne fungi concentration can lower the risk of nosocomial infection in acute care (Hashimoto and Kawakami 2018) (Abdul Salam et al. 2010)

Again, 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).

4.6. Microbial Contamination of the Air Filters

Particulate matter air pollution can affect human health. Natural protective mechanisms of the human body keep out fine dust with a size of more than 10 μm. However, the smallest fine dust particles can enter pulmonary alveoli, transfer the alveolar respiration membrane and enter the blood circulation. Excessive concentrations of fine dust in the air may exacerbate particular diseases as heart diseases, diabetes, asthma and chronic respiratory diseases (Szczotko et al. 2022). Particulate matter can be especially dangerous in the case of immune deficiency, respiratory diseases or cardiovascular problems. Young children and senior citizens are also part of the risk group.

Therefore, the air filters that are removing especially the fine particles are necessary for all means of public transport, automotive air conditioning systems,

factory level air filtration and buildings air conditioning systems.

4.6.1. 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.

4.6.2. 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).

4.7. 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.

Currently many of air conditioning systems, portable air purifiers, automotive air conditioners, commercial aircraft and other means of transport air conditioning systems use HEPA filters. Air filters (including HEPA filters) are usually made of patented filtration media. These media are based on materials containing fibers arranged perpendicularly to the air flow. Fibers are made of fiberglass, expanded polytetrafluoroethylene (PTFE) (Perry, Agui, and Vijayakumar 2016), (Shim et al. 2021). Micron-grade filter materials can consist of polypropylene (PP) or polyester as well. The main advantages of such filters are represented by high filtration efficiency and charge-based particle collection mechanism (Zhang et al. 2018). Currently modern materials as polylactic acid

(PLA) fibers are investigated (Wang, Zhao, and Pan 2015), (Zhang et al. 2019). The other biodegradable materials for air filtration as cellulose could be interesting and are also in the center of attention (Lippi et al. 2022). The filtration media are usually pleated within a framework containing some support elements or may be support-free. HEPA filters are comparably well defined in Europe and in the USA. As defined by the Institute of Environmental Sciences and Technology (IEST, USA) according to standards for air filters efficiency evaluation and testing (IEST-RP-CC001.3 and MIL-STD-282), HEPA filters must capture a minimum of 99.97 % of particles at 0.3 micrometers in size. In Europe EN 1822:2019 is binding. It defines HEPA filter as 99.995% minimum capture of particles at 0.3 micrometers in size ("EN 1822-1:2019: High efficiency air filters (EPA, HEPA and ULPA) - Part 1: Classification, performance testing, marking" 2019). Particles of this size are the most difficult to capture and thus are considered the most penetrating particle size (MPPS). The theory of MMPs is applicable under the conditions when the air flow velocity is low to moderate. At high filtration velocities, the most penetrating particle size may become substantially smaller than 0.3 μm (Lee and Liu 1980). Particles that are larger or smaller are filtered with even higher efficiency (Monto 2002), (Xu and Zhou 2014).

Theoretical explanation of particles behavior is dependent on particle-fiber interaction within the filtration fibrous material. The fiber arrangement in the HEPA filters enable several models of filtration mechanism – diffusion based on the Brownian movement of molecules, internal impaction, direct interception, and sieving (Mohammed, et al. 2022), (Wines and Mokhatab 2022). Inertial impaction is based on inertia which works on large, heavy particles suspended in the flow stream. These particles are heavier than the air flow surrounding them. As the fluid changes direction to enter the fiber space, the particle continues in a straight line and collides with the media fibers where it is trapped and held. Direct interception works on particles in the mid-range size that are not quite large enough to have inertia and not small enough to diffuse within the flow stream. These mid-sized particles follow the flow stream as it bends through the fiber spaces. Particles are intercepted or captured when they touch a fiber. Sieving occurs when the particle is too large to fit between the fiber spaces (Hinds 2021),(Boudina, Gosselin, and Étienne 2020). Diffusion is the dominant collection mechanisms for particles smaller than 0.2 μm, interception works on particles up to 0.6 μm. Particles of around 1 μm or greater may be effectively removed by inertial impaction (Tcharkhtchi et al. 2021). The mechanisms of air filtration in fiber-based filtration medium are shown in the Figure 10.

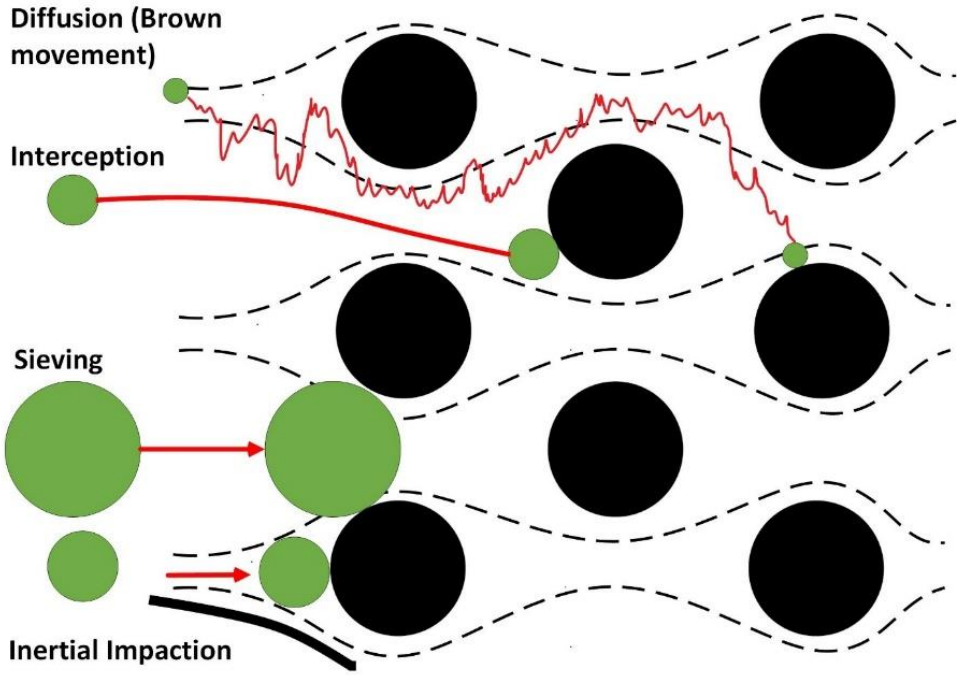

Figure 10 Filtration mechanisms. The figure represents a cross section of a fiber-based filtration medium. The interrupted black line suggests the direction of air flow. The black circles are the cross sected fibers of the filtration medium. The green objects show filtered particles. The red lines or 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).

Impaction and interception are the dominant collection mechanisms for particles greater than 0.2 μm, and diffusion is dominant for particles less than 0.2 μm (Figure 11).

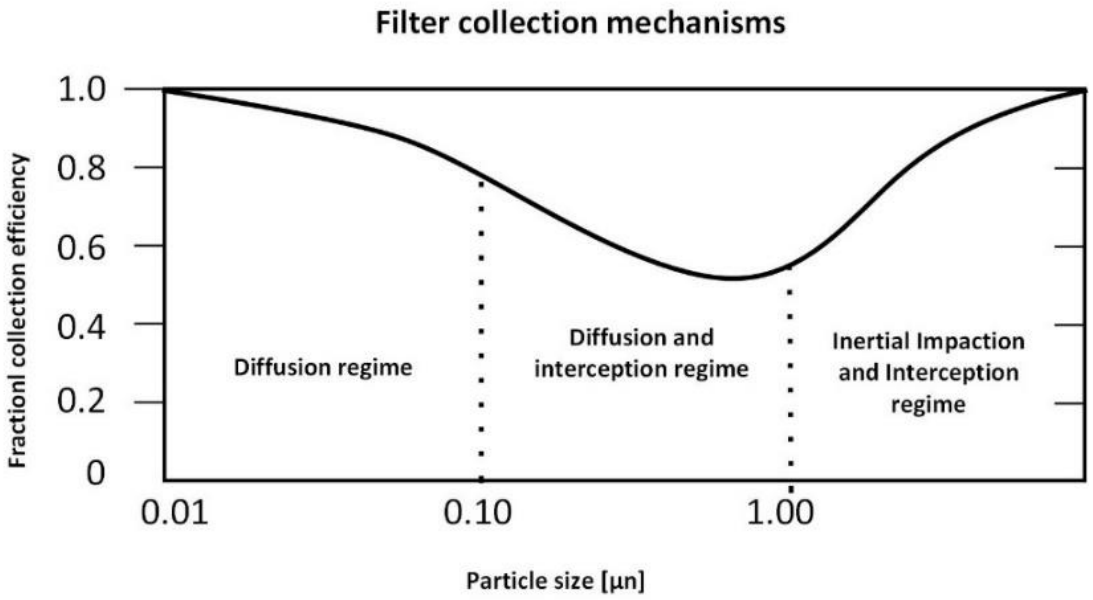

Figure 11 Fractional collection efficiency versus particle diameter for a mechanical filter axis x particle diameter (micrometer), axis y relative efficiency (National institute of Occupational Safety and Health 2003) (Daniela Obitková 2023)

As mechanical filters load with particles over time, their collection efficiency decreases and pressure drop typically increases. Eventually, the increased pressure drop significantly inhibits airflow, and the filters must be replaced. For this reason, pressure drop across mechanical filters is often monitored because it indicates when to replace filters.

When dealing with commercial aircraft cabin filtration, there are no direct and strict guidelines for filter change frequency and for filter maintenance frequency. Both depends on the filter manufacturers' recommendation and manuals for certain filter. The number of flying hours between regular service cheques of the cabin air filters can range between 500 and 6000 flying hours and depends only on aircraft type and the aircraft owner (HEPA Cabin Air Recirculation Filter).

HEPA filters are used not only in aircrafts, but also in heating ventilation air conditioning systems (HVAC) of buildings or in areas with the highest requirements on air cleanness.

Focused on the microorganisms, they differ in size considerably. The bacterial size ranges generally from 0.1 μm to 10 μm, viruses, despite they can make clusters or be bound to other particles, are usually 10-120 nm in size. According to cabin air filter producers, the cabin air filters are efficient enough in microorganism removal.

Table 4 The microorganism sizes

Microorganism	Size	Citation
Escherichia coli	0.5x1-3 μm	(Zhou et al. 2022)
Staphylococcus aureus	0.5-1.5 μm	(Gnanamani, Hariharan, and Paul-Satyaseela 2017)
Streptococcus pneumoniae	0,5-1.25 μm	(Murray, Rosenthal, and Pfaller 2016)
Staphylococcus haemolyticus	0.8-1.3 μm	(Baron 1996)
Mycoplasma pneumoniae	1-2 μm x 0.1-0.2 μm	(Saraya 2017)
Mycobacterium tuberculosis	0.2–0.5 × 1.0–1.5 μm	(Han et al. 2015)
Legionella pneumophilla	3 - 5 μm	(Percival and Williams 2014)
Bordetella pertussis	0.5-1.0 μm	(Ryan and 2004)
Neisseria menningitidis	0,6-1 μm	(Baron 1996)
Adenovirus	80-90 nm	(Desheva 2019)
Rhinovirus	30 nm	(To, Yip, and Yuen 2017)
SARS CoV-2	100 nm	(Bar-On et al. 2020)
Parainfluenza virus 1	17x9 nm	(Henrickson 2003)
Influenza A virus	80-120 nm	(Stanley 1944)
Respiratory syncytial virus	0,5-12 μm x 90-130 nm	(Norrby, Marusyk, and Örvell 1970) (Ke et al. 2018)
Cytomegalovirus	105 nm	(Ho 1982)
Aspergillus fumigatus	2.0 -3.0 μm	(Kwon-Chung, Sugui, and Heitman 2013)

Table 5 EN 1822/2021 classification of HEPA filters. H classifications are referred to as HEPA filters while U class are referred to as ULPA filters.

Filter Class	Particle Size for Testing	Collection Efficiency in %
H13	MPPS	≥99,95
H14	MPPS	≥99,995
U165	MPPS	≥99,9995
U16	MPPS	≥99,99995
U17	MPPS	≥99,999995

HEPA filters can remove at least 99.97% of airborne particles 0.3 micrometers (μm) in diameter. Particles of this size are the most difficult to filter and are thus considered the most penetrating particle size (MPPS). Particles that are larger or smaller are filtered with even higher efficiency.

In commercial aircraft as Airbus or Boeing, the mechanical air filters are used. To enhance vapor, VOCs and other chemical contaminants removal, the activated carbon is placed deeply within the pleated lamellas of the filter. The activated carbon suppresses bacterial growth and can improve the filtration capacity of the air filter. Next Table 4 brings the overview of different pathogenic or potentially pathogenic bacteria, viruses and fungi which may occur in the air or as contaminates of the air filters. Each infectious agent has different size and especially viruses may represent a challenge for air filters collection effectivity.

4.7.1. Categorization of Air Filters

The filters may be categorized according to their efficiency. The standards are different all over the world. There are standards valid mainly in Europe or in the USA. The EN standards categorize the air filters according to their efficiency in MMP's collection. So, the HEPA or ULPA (ultra-low particles air) filters could be distinguished. The ULPA filters must remove at least 99.9995% particles 0,12 μm in diameter. The Table 5 provides the summary of HEPA and ULPA filters categories.

The HEPA and ULPA filters are usually used in household air purifiers, vacuum cleaners, in the hospitals in surgery rooms, in special wards with high demands on air filtration or infectious wards. The HEPA filters are also suitable for laboratory operations – all biohazard boxes and chemical safety boxes employ HEPA filters.

The ISO standards categorize the air filters according to their efficiency in collection of particles of different sizes. Four categories are distinguished in the EN ISO 16890:

- ISO COARSE – coarse particles
- ISO ePM_{10} –particles < 10 μm
- ISO $ePM_{2,5}$ – particles < 2,5 μm
- ISO ePM_{1} – particles < 1 μm

Exact EN ISO 16890 classification is summarized in the Table 6.

The test method described in this part of ISO 16890 is applicable for air flow rates between 0,25 m3/s and 1,5 m3/s), referring to a test rig with a nominal face area of 610 mm × 610 mm.

Table 6 ISO 16890 air filter classification

Classification	Minimum filter efficiency		
	$PM_{1,\ min}$	$PM_{2,5,\ min}$	PM_{10}
ISO COARSE	–	–	< 50 %
ISO ePM10	–	–	≥ 50 %
ISO ePM2,5	–	≥ 50 %	–
ISO ePM1	≥ 50 %	–	–

In the USA the MERV classification of air filters is also used. Minimum Efficiency Reporting Value (MERV) is a North American standard that rates filters based on their ability to trap particles of different sizes. The MERV standard was issued by American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE). It is useful mainly for heating/ventilation and air conditioning (HVAC) air filters orientation. The MERV filters are mainly used for HVAC of buildings. When comparing to the HEPA filters, MERV 7 to 13 are almost as effective as true HEPA filters at removing allergens within residential air handling units (United States Environmental Protection Agency 2018). The following Table 7 summarizes the MERV classification in comparison to ISO 16890 standard.

Table 7 The MERV classification of air filters compared to ISO 16890 standard.

Class	MERV rating	ISO 16890	Controlled contaminants
Pre Filter	MERV 2	Coarse 40%	Dust mites Sanding dust Spray paint dust Textile fibers
	MERV 3		
	MERV 4		
	MERV 5	Coarse 50%	Mold Spores Hair spray Cement dust Snuff Powdered milk
	MERV 6		
	MERV 7	Coarse 90%	
	MERV 8		
Final Filter	MERV 9	ePM10 ≥50%	Pollen Lead dust Milled flour Coal dust Auto emissions Nebulizer drop Welding fumes
	MERV 10		
	MERV 11	ePM10 >60%	
	MERV 12		
	MERV 13	ePM2.5 >65%	All bacteria Cooking oil Most smoke Copier toner Most face powder Most paint pigments
	MERV 14	ePM1 70%	
	MERV 15	ePM1 >80%	
	MERV 16		

4.8. 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). In healthcare application, the nanotextiles made of nanofibers can enhance the filtration effect and protectivity for example in face masks (El-Atab, Mishra, and Hussain 2021). During COVID-19 pandemic, the nanofiber textiles have been shown to have significant potential to capture SARS CoV-2 in face masks (Naragund and Panda 2022). Among the nanotechnology products, nanofibers are one of the unique materials. Nanofibers have one order of magnitude smaller diameter than

conventional fibers. The high surface-to-volume ratio, low resistance and enhanced filtration performance make nanofibers an attractive material for many applications including air filtration. In this field the nanofibers fabricated by electrospinning technique were suggested to have unique properties (Sundarrajan et al. 2014). Electrospinning is a simple, versatile, and economical technology. In the electrospinning method, a high voltage is applied to a polymer solution to produce ultra-fine fibers ranging from several nanometers to 2 μm. Generally, horizontal or vertical setup of electrospinning apparatus can be used. electrospinning process is mainly based on the principle that strong mutual electrical repulsive forces overcome weaker forces of surface tension in the charged polymer liquid (Chew et al. 2006). Electrospinning is conducted at room temperature with atmosphere conditions. Basically, an electrospinning system consists of three major components- a high voltage power supply, a spinneret and a grounded collecting plate. It utilizes a high voltage source to inject charge of a certain polarity into a polymer solution, which is then accelerated towards a collector of opposite polarity (Bhardwaj and Kundu 2010).

When the airflow containing particles passes through the electrospun fiber felt, the air can slip on the fiber surface, resulting in a substantial loss of pressure drop before and after the airflow passes through the fiber felt. The substantial reduction is beneficial to the smooth flow of air through the filter material so that the filter performance of the nanofiber felt reaches the ideal performance. Generally, the quality factor is used to evaluate the filtration performance of filter media (Alia and Ain 2020).

Quality factor is defined as

$$QF = -\frac{\ln(1-\eta)}{\Delta P}$$

where η is the filtering efficiency and ΔP represents pressure drop. Higher QF (quality factor) can be achieved by the enhancement of filtering efficiency and the reduction of the pressure drop. Nanofiber filter media has higher filtration efficiency, and lower pressure drop than traditional fiber filter materials, that is, higher quality factor. Therefore, nanofiber filtration materials have better filtration performance (Rajak et al. 2019), (Zhou et al. 2022).

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).

The incorporation of antimicrobial agents such as silver with nanofiber is known to exhibit antimicrobial properties to the filters. Neeta et al reported antimicrobial (E. coli and P. aeruginosa) activity for poly(vinyl chloride) PVC, cellulose acetate (CA) and polyacrylonitrile (PAN) nanofiber membranes containing Ag nanoparticles (Lala et al. 2007). Some researchers added benzyltriethyl ammonium chloride to polycarbonate solution (PC) (Sun et al. 2017). Some polymers such as Chitosan (CS) and Polymethyl acrylate (PMA) have inherent antibacterial/antiviral properties usually due to their positively charged domain structure which capture and disrupt the membrane of micro-organisms. Among them, CS and some of its derivatives enjoy advantages such as relatively good antimicrobial activity, biodegradability, and non-toxicity which attract a lot of attention recently. It has also been reported that one of the sulfated derivatives of CS (sulfated chitooligosaccharide) can inhibit HIV-1, which may have potential application for medicine to control HIV infection state. There is even a report on

the effectiveness of N-[(2-hydroxy-3- trimethylammonium)propyl] chitosan chloride (HTCC) on inhibition of the new coronavirus or SARS-CoV-2 (Borojeni, Gajewski, and Riahi 2022).

5. 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.

5.1. 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.

5.2. 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.

5.3. 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.

5.4. 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. ISO EN 18593 describes the following workflow:

- Material and equipment. The standard describes the appropriate equipment as breakable sticks with cotton or synthetic material swab contained in a tube or envelope. The swabs shall be individually wrapped and sterilized. The material used shall be documented free of inhibiting substances. The swabs are suitable for difficult accessible places rich in slots or cracks.
- Sampled area. The sampled surface shall be as large as possible, if it is accessible. For microorganism detection the sampled surface shall be 1000-3000 cm2. For microorganism quantification the square of 100 cm2 is sufficient.
- Swabs. Depending on the nature of the sampled surface, both wet and dry swabs are recommended. To prepare the wet swabs, the tip of the stick is moistened with sterile saline, neutralizing substance or cultivation medium. Rotation of the swab is recommended to obtain the optimal amount of the sample.
- Microbiological analysis. To the used device, the appropriate amount of dilution solution/liquid cultivation media is added. Recommended volume is referred to 9-10 ml. The swab shall be rinsed in the solution thoroughly to obtain the sample for subsequent procedures.
- Results expression and calculation. When rinsing the swab in 10 ml of solution mentioned above, the specimen for logarithmical dilution is prepared. The qualitative results are expressed as the name of detected microorganism ("ČSN ISO EN 18593: Mikrobiologie potravinového řetězce - Horizontální metody specifikující techniky vzorkování z povrchů" 2019).

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. The Gram stain was performed as listed in the Table 8.

Table 8 Gram stain schema - adapted according to (Leboffe and Pierce 2011)

Dye	Time [s]	Note
Crystal violet	60	All bacteria in the specimen are stained – purple color
Lugol's iodine solution	30	
Acetone or alcohol	15	Destaining step: G+ bacteria keep the purple color G- bacteria get destained
Carbol-fuchsine or safranin	60	G- bacteria get pink color

5.4.1. 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).

Catalase test. Catalase is the enzyme that breaks H_2O_2 into O_2 and H_2O. All microorganisms living in oxygenated atmosphere need mechanisms to get rid of toxic oxygen metabolites. Positive result of this test - presence of the enzyme, lies in O2 evolution (bubbles production) after addition of H2O2 to the bacterial specimen. The purpose of the test is to distinguish between Staphylococci which are catalase positive and Streptococci that are catalase negative.

Oxidase test. This test is used to determine the presence of bacterial cytochrome oxidase which indicates aerobic metabolism of the microorganism. Under aerobic condition, in the respiratory chain, the enzyme cytochrome oxidase transfers electrons to the terminal acceptor represented by oxygen. During the test

the substrate N, N-dimethyl-1, 4- phenylendiamin serves as an acceptor of electrons and the change of color will occur indicating the presence of cytochom oxidase enzyme.

A strip having a test zone impregnated with N, N-dimethyl-1, 4-phenylendiamin is applied to the surface of bacterial colony. The substrate N, N-dimethyl-1, 4- phenylendiamin is converted to indol blue after incubation which indicates positive reaction.

Entero test 16. This kind of biochemical test is designed to differentiate the *Enterobacteriacae*. The test consists of 16 biochemical parameters including sulfan, lysin, indol, ornithin, urease, fenylalanin, aesculin, Simmons citrate, malonate, inositol, adonitol, celobioze, sacharse fermentation, sorbitol fermentation, trehalose, mannitol. The bacterial specimen is suspended in 3 ml sterile saline. The particular reactions are performed in the wells of microtitration plate where 0,1 of bacterial suspension is inoculated to each well. The results are available after 18-24 hours incubation at 37°C and are evaluated in accordance with the color scale table enclosed to the test. The outcome is converted to the number value which corresponds to the particular organism (Říhová Ambrožová and Trögl 2014).

Coagulase test. Coagulase test is used to differentiate *Staphylococcus aureus* (positive) from coagulase Negative Staphylococci. Coagulase is an enzyme produced by S. aureus that converts fibrinogen in plasma to fibrin. *Staphylococcus aureus* produces two forms of coagulase, bound and free. While the free coagulase is an enzyme secreted extracellularly, the bound one is cell wall associated protein. Slide coagulase test is done to detect bound coagulase (clumping factor). Tube coagulase test is done to detect free coagulase.

Principle and procedure:

a) Clumping factor test – two spots of dense suspension of bacterial cultures are made on opposite sides of clean glass plate. One test and one control spot. One loopful drop is applied to one suspension spotted on the slide. Agglutination of the suspension is referred as positive reaction.

   Latex agglutination representing other arrangement of bound coagulase test is usually commercially available and widely used. The procedure of the test is as follows. The test utilizes colored polystyrene latex particles sensitized by fibrin and IgG. When staphylococcal colonies which possess bound coagulase are mixed with the latex reagent, the latex particles agglutinate usually within 20 seconds (Pro-Lab Diagnostics).

b) Free coagulase test – three test tubes labeled as test, positive control and negative control are needed. In each of these test tubes the rabbit plasma is measured off. Then 0,1 ml of 24 hours culture of tested species, known S. aureus and sterile cultivation media free from bacteria is applied to the plasma in test tubes. All test tubes are incubated at 37 °C up to 4 hours. Gellification of plasma is considered as positive reaction.

CAMP test. CAMP test is usually used to identify group B *Streptococci*. The principle of test is based on the synergic effect of β-hemolysin of *St. aureus* and CAMP factor of *Streptococci* which enhances lysis of red blood cells in agar plate. Streptococcus agalactiae has positive reaction, *Streptococcus pyogenes* gives negative reaction.

5.4.2. 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:

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

5.5. 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, so the maximum score is 3. The minimum score for reliable detection amounts to 2 and 3.

5.6. 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).

5.6.1. 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 9.

Table 9 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 10.

Table 10 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 11.

Table 11 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 12, 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.

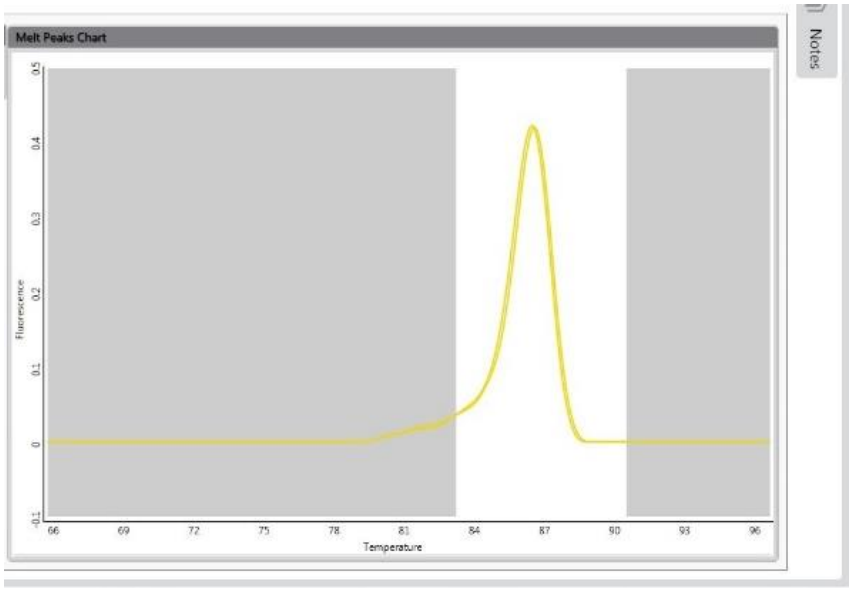

Figure 12 The peak of Seratia marcescens obtained by FilmArray 2.0, Pulmonary panel (Daniela Obitková 2019)

5.6.2. 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 12.

Table 12 The Respiratory SARS CoV-2 Panel (Producers' manual to the Respiratory panel)

Pathogenic agent	Classification
Influenza A	Orthomyxovirus (RNA)
Influenza A (subtype H1N1/2009)	
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	

6. 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 13. 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 13 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

6.1. 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 13 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 13, 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.

6.1.1. 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.

6.1.2. 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.

6.1.3. 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 14 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 15 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 14 and Table 15.

6.1.4. 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 16. It is clearly visible that the outlet surface is ten times more contaminated than the inlet surface of the filter.

Table 16 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.	$3{,}2 \times 10^6$	$1{,}63 \times 10^7$

6.2. 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 17, the dimensions of air filters are listed. The table also contains the size in square centimeters.

Table 17 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 14 represents the cultivated *Bacilli* on blood agar plates. In the filter 17, on the inlet side *Brevibacillus laterosporus* was identified as well.

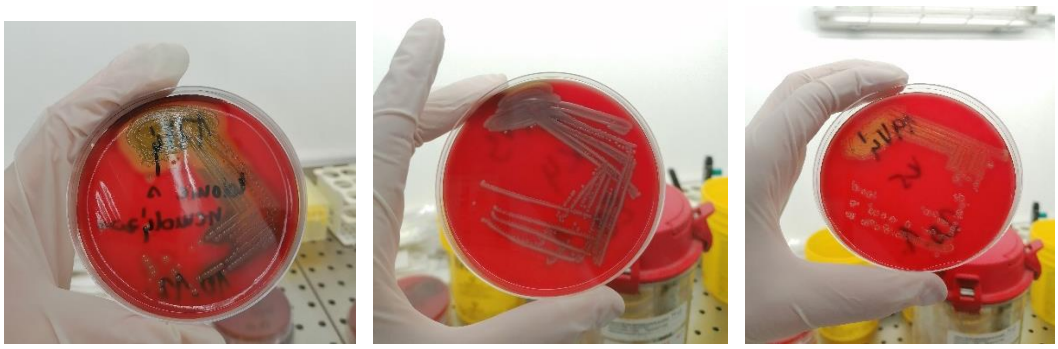

Figure 14 Blood agar plates with 24-hours cultures of detected bacteria. The first picture refers to the culture of *Bacillus cereus*, in the middle the culture of *Bacillus subtilis* is situated and on the right side the culture of *Bacillus licheniformis* is depicted (Daniela Obitková 2021)

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 18 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 19 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 20 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 21 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 22 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 23 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

6.2.1. 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 24 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 24.

Table 24 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.

6.3. 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 25 represents the dimensions of all investigated filters.

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

Table 25 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 26 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 27 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 28 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 29 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 30 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 31 Quantification of bacteria in the filter 3 represented as CFU/cm^2

	inlet [CFU/cm^2]	outlet [CFU/cm^2]
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 32 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 33 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 34 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 35 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 36 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 37 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 37 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.

6.4. 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.

6.4.1. 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. The procedure of the single run is presented in the Table 38.

Table 38 Summary of procedures within automated run: adapted according to datasheet of Respiratory and Pulmonary panels (BioFire Diagnostics LLC 2017), (BioFire Diagnostics LLC 2018)

Lysis of the sample by agitation (bead beading)	
Nucleic acid extraction and purification (magnetic bead technology)	
Nested multiplex PCR	• First performing reverse transcription and a single, large volume, massively-multiplexed reaction (PCR1) • Then performing multiple singleplex second-stage PCR reactions (PCR2) to amplify sequences within the PCR1 products
endpoint melting curve data are the basis for particular target detection	
real-time PCR data used to provide semi-quantitative information about number of nucleic acid copies (bacterial targets in Pneumonia Panel only)	

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 15). 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.

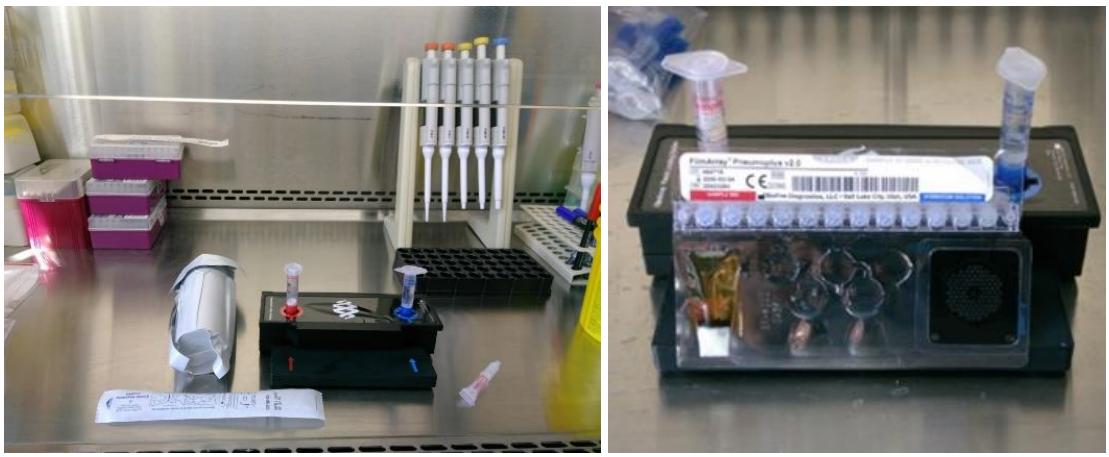

Figure 15 The FilmArray kit in the laminar-flow class II biosafety cabinet, the assembled loading station with reagent pouch, hydration injection vial (blue) and sample injection vial(red) (Daniela Obitková 2019)

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. The workflow of both Respiratory and Pulmonary panels is presented in the Table 39.

Table 39 Respiratory and Pulmonary Panel workflow

Respiratory panel	Pneumonia Panel
sampling – dry Dacron swabs rinsed in saline or Remel transport medium included in Microtest TM M4RT® Kit: Swabs: 2 Female (Remel Europe Ltd)	sampling – damp flockswab with breakable shaft (Copan) of the surface of the filter dilution solution – sterile saline
unpacking of the kit	
loading station assembly – the reagent pouch and the vial with moisturizing solution and sample injection vial were inserted to the loading station	
moisturization of lyophilized reagents within the pouch	
sample buffer loading to the sample injection vial	
200 μl of prepared specimen added to the sample buffer	the flockswab with specimen inserted to the sample buffer, break of the shaft and closure of the tube
injection of the mixture (sample buffer + specimen) to the pouch	
loading of the pouch to the Filmarray 2.0 instrument for automated processing	
~ 1 hour	
results	

6.4.2. 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 16 depicts the Respiratory panel cartridge.

Figure 16 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 17.

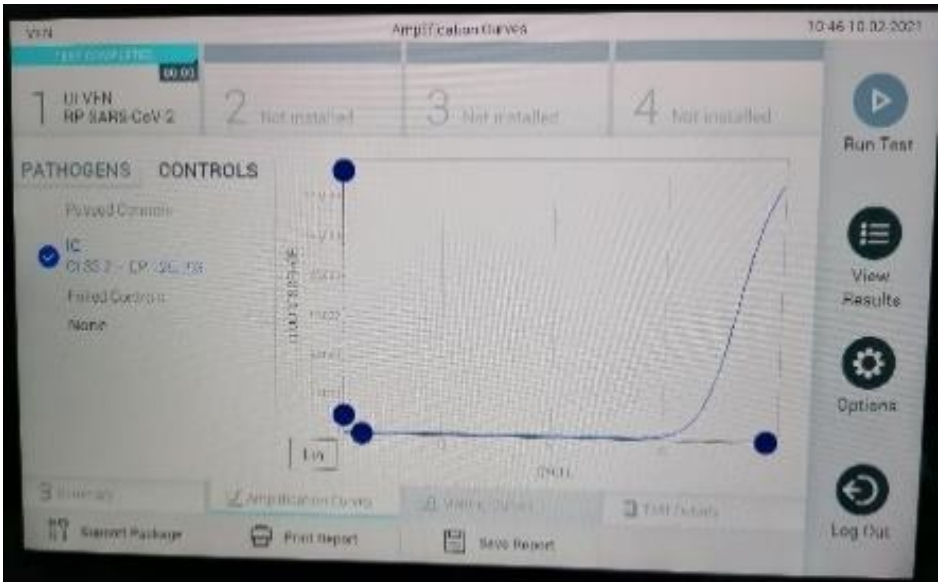

Figure 17 The PCR curve of internal control of QIAStat DX® Respiratory SARS CoV-2 Panel (the x axis represents time, the y axis summarizes the number of PCR cycles).

6.4.3. 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.

6.4.4. 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.

6.4.5. 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 18.

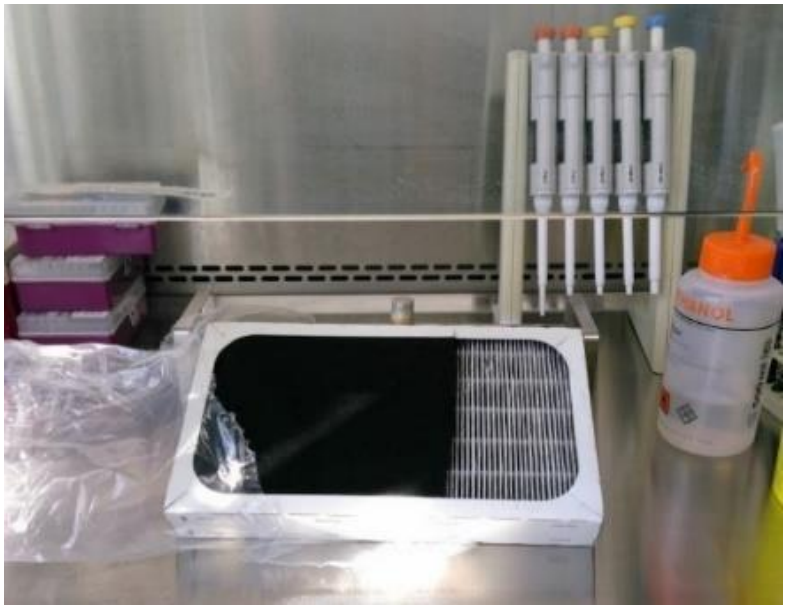

Figure 18 The HEPA filter removed from household air condition (Daniela Obitková 2019)

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 40.

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

Table 40 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

6.4.6. 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 41.

Table 41 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 42.

Table 42 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

6.4.7. 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 19.

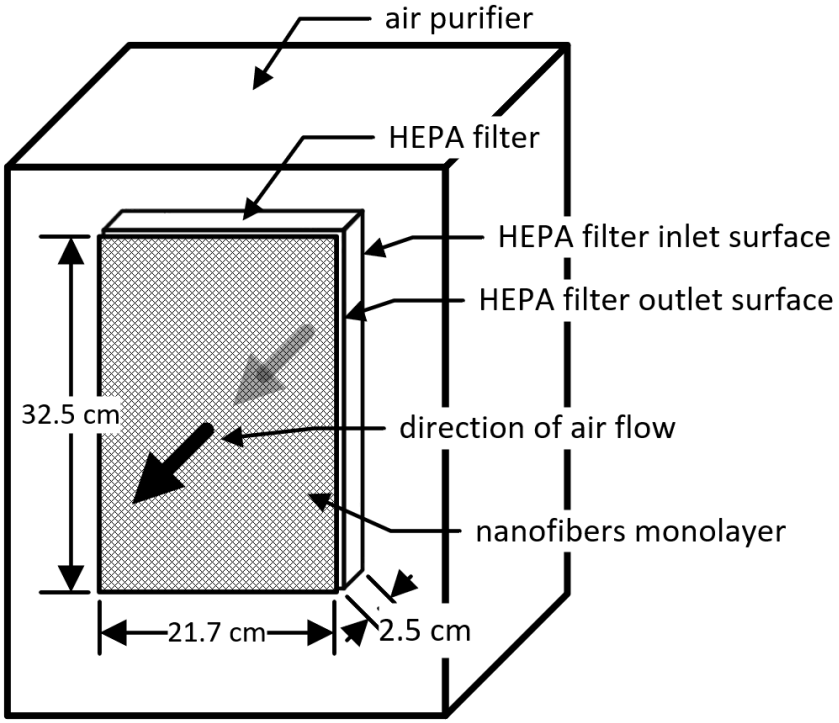

Figure 19 The household purifier experimental setup.

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 43). 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 43.

Table 43 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

7. Discussion

Air filtration is an urgent global need because in many countries and regions the high concentration of inhalable suspended particles in the air is causing irreversible damage to human health. Particulate matter (PM) occupies a prominent position among the major pollutants in the atmosphere. It is considered one of the six criteria air pollutants classified by the U.S. Environmental Protection Agency (USEPA). PM is a term that describes a mixture of particles of various shapes and sizes that enter the atmosphere from a variety of sources, including the combustion of fossil fuels, industrial emissions, dust, smoke, and fog. These particles can range from coarse to fine, with diameters varying from fewer 10 μm to sub-micrometers. Particulate matter (especially $PM_{2.5}$) poses a significant risk to human health as it can penetrate deep into the respiratory system when inhaled, leading to respiratory infections and exacerbating heart and lung diseases. A recent United States Environmental Protection Agency (USEPA) report has shown that nearly 2.1 million deaths worldwide occurred due to high $PM_{2.5}$ concentration. World Health Organization (WHO) report, air pollution is responsible for seven million people's death worldwide every year(World Health Organization 2023) .

$PM_{2.5}$ penetrate deeply to the lung alveoli and can be accumulated causing different respiratory diseases as well as lung cancer (Lu et al. 2015). The $PM_{2.5}$ has a higher toxicity than PM_{10} due to the inflammation-causing capacity and oxidative stress (Valavanidis, Fiotakis, and Vlachogianni 2008). Oxidative stress may lead to exacerbation of bronchial asthma, coronary artery disease or contribute to other chronic respiratory diseases development. The health risks of $PM_{2.5}$ is the reason why majority of means of transport and building air conditioning systems are equipped by the air filters intended for elimination of particulate matter of this particulate size. Regarding microorganisms, the bacteria and especially viruses are significantly smaller. The bacteria can settle on coarse dust (PM_{10}) and the chance to eliminate them from filtered air could be higher. To eliminate viruses, we should take into regard economic and technical data. HEPA filters or nanofibrous filters could be an optimal choice, but especially in the case of HEPA filters the pressure drop behind the filter is too high and cannot meet the technical demands of selected air conditioning systems. This situation is applicable especially in urban buses – the air conditioning system cannot operate with HEPA filter and moreover frequent door opening brings high doses of new dust and particulate matter what can cause ultimate clogging of the filter.

7.1. Indoor Air Quality Standards

Since 2020, the annual average concentration of $PM_{2.5}$ in ambient air is limited to 20 μg/m^3 in European Union (European commission 2012). This limitation was imposed because of the observed health impacts associated with the various pollutants' occurrence over different exposure times. Under EU law, a limit value is legally binding from the date it enters into force, subject to any exceedances permitted by the legislation. In the Czech Republic the limits for $PM_{2.5}$ were equalized to the European Union standard since this year(*Zákon č. 201/2012 Sb. Zákon o ochraně ovzduší* 2012). World Health Organization recommends to the states all over the world to decrease $PM_{2.5}$ emissions. WHO issued WHO global air quality guidelines where the air pollution is now recognized as the single biggest environmental threat to human health. The WHO recommends decreasing the limits for annual average of $PM_{2.5}$ to 10 μg/m^3 (World Health Organization 2021). Unite states Environment Protection Agency limit for annual average $PM_{2.5}$ concentration is 12 μg/m^3 (United States Environmental Protection Agency 2012).

$PM_{2.5}$ concentration in ambient air is strictly limited due to their serious impact to human health. The same situation is in the indoor spaces where the indoor air quality is also monitored and the ventilation and air conditioning systems of buildings and transportation vehicles are mainly equipped by $PM_{2.5}$ filters as we can see in automotive and bus air conditioning systems. So, the air filters for ground transportation are chosen well and in accordance with current knowledge and standards. The problem of $PM_{2.5}$ could be in their capability to carry dangerous chemical agents or toxic substances as heavy metals (Sakunkoo et al. 2022). The bacteria and can be also present in dust particles, this is primarily known about environmental genera of bacteria as *Bacilli*, *Brevibacilli* or *Clostridium*.

In the Czech Republic the indoor concentration of microorganisms is limited and controlled only in public buildings as schools, universities, health care centers of any purpose, commercial accommodation and social care centers. The given limit for microorganism concentration indoors cannot exceed 500 CFU per m^3 of the air. The EU limits the microorganism's concentration according to the EUR 14988 EN. The standard applies for homes, public buildings and non-industrial buildings, excluding hospitals. The given limit of microbial concentration in household is 2500 CFU per m^3 (Rubinová, Počinková, and Raputa 2016). In the Czech Republic, the limit for households is not given exactly. The means of transport in the Czech Republic do not have any limits of microbial concentrations as well. In our experiments we investigated the microbial contamination of air filters and the sufficiency of conventional air filters in microorganisms'

elimination. The examination of air microbial burden would be worth to suggest the microorganism concentration in the means of transport, especially in enclosed cabins of aircrafts, trains, subways of coach buses. Interestingly, the concentration of microorganisms could be correlated with minimum infective doses for selected pathogens to estimate the danger of particular microorganism concentration (SeyedAlinaghi et al. 2022), (Yezli and Otter 2011).

7.2. Population Protection

Health studies have shown a significant association between exposure to particle pollution and health risks, including premature death. Health effects may include cardiovascular effects such as cardiac arrhythmias and heart attacks, and respiratory effects such as asthma attacks and bronchitis. Exposure to particle pollution can result in increased hospital admissions, emergency room visits, absences from school or work, and restricted activity days, especially for those with pre-existing heart or lung disease, older people, and children. $PM_{2.5}$ is mostly absorbed through the respiratory system, where it can infiltrate the lung alveoli and reach the bloodstream. In the respiratory system, reactive oxygen or nitrogen species (ROS, RNS) and oxidative stress stimulate the generation of mediators of pulmonary inflammation and begin or promote numerous illnesses. Particulate matter (PM) is made up of solid and liquid particles that are discharged directly into the air as a result of diesel use, road and agricultural dust, and industrial activity. Various chemicals were found as components of $PM_{2.5}$. In the aerosols originated in the sea, sodium (Na) can be found. In $PM_{2.5}$ of industrial origin Iron (Fe), Zinc (Zn), Copper (Cu), Lead (Pb), Nitrates (NO_3) are present. The soil and dust contain Aluminium (Al), Silicon (Si), Calcium (Ca). Volatile organic compound (VOC) emissions Benzene, Ethylene glycol, Formaldehyde, Methylene chloride, Tetrachloroethylene, Toluene, Xylene, and 1,3-Butadiene could be also components of PM2.5. The lungs, the initial sites of $PM_{2.5}$ deposition in the airway, are among the primary targets of $PM_{2.5}$-induced toxicity, which leads to airway inflammation, bronchial asthma, chronic obstructive pulmonary disease, lung cancer, impairing normal immune responses of the lungs and making them susceptible to various respiratory infections. It has been hypothesized that $PM_{2.5}$ impairs the normal immune responses by various mechanisms. Firstly, $PM_{2.5}$ can damage the bronchial mucociliary system, reducing bacterial clearance. Recently, increasing evidence has shown that $PM_{2.5}$ not only inhibits alveolar macrophage phagocytosis by disrupting the normal physical and immunological function of

lung surfactants, but they also impair the response of natural killer (NK) cells and inhibit antibacterial capabilities (Thangavel, Park, and Lee 2022). $PM_{2.5}$ may also contain various microorganisms. The sources of the microbes are generally two – firstly, the nature with strongly resistant bacteria pathogenic potentially pathogenic or non-pathogenic for humans. Secondly, the humans are the source of pathogens or commensals typical for different organ systems. Indoors, in the air, surfaces and settled dust different bacteria were identified. The most common bacterial genera observed in dust and air samples collected from various types of indoor environments are *Acinetobacter*, *Actinobacteria*, *Arthrobacter*, *Alcaligenes*, *Bacillus*, *Corynebacterium*, *Kocuria*, *Micrococcus*, *Propionibacterium*, *Staphylococcus*, and *Streptococcus* species. The fungal genera predominating various indoor environments are *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium*, *Stachybotrys*, *Trichoderma*, and yeasts like *Candida* spp. It has been shown that the indoor air of residential apartments in South Korea mainly contained adenoviruses and influenza A virus (Chawla et al. 2023). In our experiments with household air purifier we found adenovirus in the HEPA filter of the air filtering device too. The space of cabins of means of transport, especially the aircrafts revealed similar bacteria as in the studies mentioned above. In this field, our results are in accordance with other studies made worldwide.

In the Czech Republic the population health and hygienic standards are regulated and controlled by legislation. As mentioned above, the $PM_{2.5}$ concentrations in ambient air are given in the law of ambient air protection. Hygienic standards for various indoor environments are regulated by the ministerial regulation number 6/2003 which gives the hygienic demands regulating concentration of particulate matter, chemicals and microorganisms in indoor air. This regulation only omits homes and clean areas. The ministerial regulation (Ministry of health care 6/2003) says that the concentration of microorganisms indoors must not overcome 500 CFU/m^3 of air (*6/2003: Vyhláška, kterou se stanoví hygienické limity chemických, fyzikálních a biologických ukazatelů pro vnitřní prostředí pobytových místností některých staveb* 2003). If the means of transport in the Czech Republic, especially aircraft and trains intended for long distance routes and coach buses could have similar regulations, it would be possible to decrease the microbial burden of the air in the means of transport. The cleaner air then could have positive impact to passengers' comfort and could support striving for improvement of public health – based on elimination of exacerbations of diseases as asthma, chronic pulmonary obstructive disease or chronic bronchitis after travelling for long distances. The regulation of microbial burden in the air in

transportation could have also impact on the health of different age groups of passengers as small children and seniors who are most susceptible to microbes' exposure. In European Union the EUR 14988 EN regulates the concentration of microorganisms indoors (except hospitals). So, when regarding the European airlines, and international ground transportation, it could be also suggested to admit selected regulations to improve public health by decreasing of microbial concentrations onboard and in the ground means of transport. Reduction of microorganisms in transportation could have positive impact not only to the health status of passengers but moreover, the healthier people will not need often medical checks or drugs prescription. All these facts may have influence in the economics of the health care system as well. Decreasing the health care costs may seem to be a good benefit.

Currently, based on the Security strategy of the Czech Republic, the armed conflict in Ukraine represents the major threat for the state stability and democracy. This conflict has broken the peaceful environment of Europe and threatened the fragile stability in economics, democracy and law. The Russian Federation has broken the chemical weapons prohibition and may abuse also progress in biological research for military purposes. People's Republic of China sympathizes with Russian Federation, so China may represent the same threat when considering chemical and biological weapons (*Bezpečnostní strategie České Republiky* 2023). When considering the biological weapon attack, we can only estimate whether the conventional air filters of buildings or means of transport would be able to capture the infectious agents. According to our experiments the HEPA filters may be sufficient to capture the bacteria, but this type of air filters may not be sufficient in viral particles filtration, especially in the case of small viruses – in our experiments the coronavirus passed the HEPA filter. The $PM_{2.5}$ air filters in our experiments showed significant insufficiency in bacteria capturing, especially in the case of bus air filters. In several parts of the world, the terroristic attacks of chemical weapons have been encountered. In the environment of military conflict in the eastern Europe or in Izrael, the attacks by biological weapons may be supposed. Biological warfare agents may be more potent than conventional and chemical weapons. During the past century, the progress made in biotechnology and biochemistry has simplified the development and production of such weapons. In addition, genetic engineering holds perhaps the most dangerous potential. When regarding the most potential biological warfare (Rift Valley fever virus, Tickborne encephalitis virus, *Salmonella typhi*, *Brucella abortus*, *Coxiella burnetii*, *Francisela tularensis*, *Bacillus anthracis*), majority of them

belong to rather smaller organisms – the viruses or F. tularensis especially (Azaki et al. 2019). Only *B. anthracis* is rather big when comparing it to other items of the list mentioned above. *B. anthracis* is or was used by terroristic attacks or in armed conflict. *F. tularensis* was used in the World War II. After the year 1995 the Russian Federation dislocated the biological weapons development to Stepnogorsk in Kazakhstan, producing the more virulent *B. anthracis*. Some information exists that in 80s', the former Soviet Union scientists worked on weaponization of smallpox virus, but nowadays little information is known about outcome of these experiments. The biological weapons were also used in the War in the Persian Gulf in the year 1991. In the USA, the offensive biological weapons program was terminated by President Nixon by executive orders in 1969 and 1970. The USA adopted a policy to never use biological weapons, including toxins, under any circumstances (Riedel 2017).

In the year 1972 the "Convention on the Prohibition of the Development, Production, and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction" was developed. This treaty prohibits the development, production, and stockpiling of pathogens or toxins in "quantities that have no justification for prophylactic, protective or other peaceful purposes". The former Czechoslovak Socialistic Republic ratified the Biological Weapons Convention (BWC) in the year 1975 by the ministerial regulation 96/1975. The Czech Republic belongs to the contracting states and involves the BWC to its legislation. The crucial law is represented by the law no. 281/2002 (The law about selected measures dealing with biological and toxin weapons prohibition) (*Č. 281/2002 Sb.Zákon o některých opatřeních souvisejících se zákazem bakteriologických (biologických) a toxinových zbraní a o změně živnostenského zákona* 2002). Currently, the Meetings of Experts a Meeting of State Parties take place and the specialist discuss the topics dealing with infectious diseases incidence, surveillance, biosafety and biosecurity. The Czech Republic nor The Czechoslovak Socialistic Republic never had developers the biological weapons.

According to The Safety strategy of the Czech Republic, Protecting the lives and health of the population not only in the event of a pandemic is fundamental obligations of the state. The Ministry of Healthcare attempts to create a resistant and strong health care system which must be prepared to admit a large amounts of patients. Each single individual is supposed to contribute to the biological safety of the Czech Republic (*Bezpečnostní strategie České Republiky* 2023).

7.3. Aviation

Investigation of the bacterial contamination of the aircraft air filter revealed significant contamination of both surfaces – the inlet and outlet. The contamination of outlet side of the filter was remarkably higher (10x) than in the inlet side. These results are in contradiction with the assumption that the outlet side of the filter should remain clean. As referred previously, the air quality within the cabin of commercial aircraft does not differ significantly from the air quality of other air-conditioned areas. The air filters used in commercial aircraft are of the same quality as in hospitals or special laboratories. Other studies, investigating removal of bacteria by the air filters proved good filter function and sufficient bacteria interception with clean air production (Mittal et al. 2011). The producers of the air filters for aircraft use (PALL Corporation, Purolator Facet Inc.) assure the public that the filtration effect of their air filters is sufficient.

Our experiments suggest poor bacterial entrapment and potential pathogen release to the cabin air and their recirculation in the cabin air. Even the larger bacteria as *Staphylococci* or *E. coli* were isolated from the outlet side of the filter with potential recirculation in cabin air. Moreover, the bacteria seem to live in the filter for very long time. The investigated filter served for 4800 flying hours, but the bacteria were cultivated and recovered after several weeks during the experimental period. Because there are no rules for service checks of the cabin air filters, the owners of the aircraft only follow the producers' instructions for use. The recommended lifetime of particular air filter is about 5000 flying hours. Our results suggest that this period could be too long. The pores obstruction and filter saturation by particles of different origin led to ultimate loss of air filter function. Shortening of air filters' change may significantly improve the filter function. It may be interesting to investigate the microbial contamination of cabin air filters of various flying hours of use. The information could contribute to balance filter safety and cost related to more frequent filter change off.

The results showing the strong microbial contamination of outlet surface of the cabin air filter may suggest the potential high health risk for passengers. Microbes recirculating in the cabin air could easily infect the persons onboard. The health risk of stay in enclosed air-conditioned space is enlarged by several additional conditions connected with air travel. According to the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE) standard, the humidity aboard should be 20% which is much lower than in the other areas. This parameter influences the comfort of the individual, breathing, motility of cilia in the respiratory epithelium and mucus transport. Dry air

dehydrates the mucus produced by the respiratory epithelium that results in cilia paralysis and mucus removal failure. Worsening of removal of the mucus content of the airways results in prolonged stay of microbes intercepted in this mucus and significantly risen the risk of infection. Despite the discomfort which is usually caused by low level of humidity, the higher amount of water vapor contained in recirculated air could cause spores and fungi recovery in the filter surfaces. To diminish this kind of risk, the humidity is maintained very low.

As reported previously, the air crew involved in aircraft air quality studies described symptoms related to decreased air humidity. The symptoms that were more frequently experienced during flights, whose durations ranged from 1 h and 25 min to 14 h and 15 min, were dry itchy or irritated eyes, dry or stuffy nose, skin dryness or irritation (Lee et al. 1999). As reported by Lee et al. the humidity in long-haul flights were the lowest when comparing with the short and medium haul flights. The immune system changes due especially long-distance flights may contribute significantly to high risk of getting infected during the flight or within several hours after landing. The long-haul air travel (more than 3 hours) causes the immune system downregulation. The immune system gets in suppression onboard. Especially the activity of TH1 cells is diminished and the start-up of cellular immunity is then decreased. This downregulation lasts several hours after landing. This time period between immune system downregulation and recovery represents the highest risk interval for development of infection gained onboard (Rose et al. 1999). The pressurization of the cabin simulates the high-altitude environment where the mild hypoxia can occur. In sensitive individuals, this mild hypoxia can augment the immune system dysfunction.

The biggest challenge for air filters is represented by viruses. Being much smaller than bacteria, the viruses probably penetrate the conventional aircraft air filters completely. In accordance with this statement, the aircraft air filter did not contain the viruses. It may be caused by filter insufficiency to stop ultra fine particles or degradation of all viral nucleic acids by ubiquitous DNases and RNases. The cause of our unsuccessful virus search can be also in swabbing technique. We did not want do dismount the filters to preserve of our conception to swab outlet and inlet surface separately. Other research team removed the frame of the filter, prepared defined samples of the filtering media and finally performed a lavage of the filtering medium. The lavage liquid acquired from 48 used aircraft air filters underwent multiplex PCR tests. Only three samples were positive. Rhinovirus, Influenza A and Influenza B were detected (Korves et al. 2011). The results show that the viruses in the aircraft air filters are very rare. So,

in our experiment testing one filter, the amount of viral nucleic was too small or there were no viruses at all.

Our experimental setup with separate investigation of the inlet and outlet surface of the air filter is unique because we are capable to evaluate the microbial contamination especially in outlet surface of the filter. This information could be valuable for estimation of air filter lifespan and potential recirculation of the microbes back to the aircraft cabin where the pathogens may threaten passengers' health. It was reported previously that the crew and passengers show symptoms of common cold, fatigue or flu symptoms after long haul flights (Coste et al. 2007)

Aircraft cabins may be high-risk environments for transmission of infectious diseases. Space confinement, limited ventilation, prolonged exposure times, and recirculating air, all common to air travel, have been demonstrated to be risk factors for transmission of upper respiratory tract infections in other settings. Several case reports detail outbreaks of influenza and tuberculosis aboard aircraft, but it is not known whether air recirculation increased rates of transmission. ASHRAE reports that the recirculation of the cabin air and high speed of recirculating air enhances the viability of virions occurring in the cabin air. The virions which cannot settle down and die off by drying or cannot be decomposed by enzymes present in the ambient air. The recirculation was found as a risk factor for infectious disease transmission. But the study comparing occurrence upper respiratory tract infection in passengers of the aircraft with recirculation and without recirculation respectively. In this study the numbers of passengers reporting upper respiratory tract infection after long haul flight did not show any difference between the group of recirculating aircraft and the aircraft without recirculation (Zitter 2002).

7.4. Automotive Airconditioning System

The detected bacterial species ranking among the members of genus *Bacilli* and *Brevibacilli* are environmentally ubiquitous. Some of them, especially *B. licheniformis* serve as natural decomposers. So, the contamination of air filters with these particular bacilli is obvious. Regarding the difference between quantity of detected microbes on inlet and outlet surfaces we can estimate that the bacteria flowing through the filters along with the filtered air can pass the filter. It means that the filter could not be efficient in bacteria interception as was suggested by the investigation of commercial aircraft filter, which showed higher bacterial contamination on outlet side of the filter (Pavlík et al. 2019).

The fact that no human pathogenic bacteria were detected could be caused by diminished resistance of pathogens to external environment. The bacteria commonly present in dust or soil show higher natural resistance to temperature or humidity changes. The pathogens could be more sensitive to UV beams or desiccation (Mittal et al. 2011). The *Bacilli* and *Brevibacilli* do not represent any danger for the private cars' owners.

Low bacterial contamination, absence of pathogenic bacteria and the detection of no viruses could be caused also by the specimen of the air filters. The personal cars are usually used by limited number of persons who travel by cars usually in healthy state. We have supposed presence of human commensals as *St. epidermidis*, *E. coli or* oral *Streptococci* or *Neisseria*. As mentioned above, these bacterial species show limited environmental survival, so we were not able to detect any cultivable individuals. Considering pathogens ubiquitous in the soil, *Clostridium tetani* or *Clostridium perfringens* could be detected. Nevertheless, the *Clostridia* were detected only in the aircraft air filter.

The biggest challenge for air filters is represented by viruses. Being much smaller than bacteria, the viruses probably may penetrate the automotive air filters. There are several factors that could explain that no viruses were detected. Presence of DNases and RNases in the environment can cause complete degradation of the viral nucleic acids searched by the PCR methods. Regarding the size of viruses, there is a possibility that the viruses pass through the filter and can recirculate in the space treated by the air conditioning system.

In addition, the automotive air conditioning systems are not exposed to high microbial burden. We investigated the filters of private cars, not for example taxis where the fluctuation of persons is very high. The number of persons occupying the cabin of the private cars is nearly constant for quite long time period. So, the "microbiota" of the car cabin seems to be stable and a number of pathogens is perhaps very low.

The situation could be completely different in company cars or in taxis. Many questions dealing with air filters efficiency have arisen during the COVID-19 disease pandemic. The tram, bus and taxi drivers were found to have the second highest risk of acquiring of COVID19 disease during the first and second wave of COVID-19 pandemic in Norway (Magnusson et al. 2021). In the UK, the Office for National Statistics (ONS) reported that road transport drivers had higher mortality rates related to COVID-19 among men; overall working age men had a mortality rate of 31.4 deaths (per 100,000), while taxi drivers and chauffeurs had a rate of 101.4 and bus and coach drivers of 70.3 (Gartland et al. 2022). These

infections were caused by transmission of SARS-CoC-2 by direct contact with passenger's bioaerosols. But there is a significant presumption that the SARS CoV-2 an recirculate via the air conditioning system. Coronavirus 229 E penetrated in our experiment the HEPA filter so, The SARS CoV-2 which is of the same diameter as C. 229E, may penetrate the $PM_{2.5}$ easily and the viral load of the taxi cabin may be significantly higher. In our tests of automotive air filters no viral nucleic acids demonstrating virus presence has been detected. It may be caused by ubiquitous DNases and RNases, but we suppose based on the experiments, that the viruses penetrate the $PM_{2.5}$ filters due to their small dimensions.

The COVID-19 pandemic has demonstrated the real need for mechanisms to control the spread of airborne respiratory pathogens. As shown by our experiments with nanotextile material, one solution could be to add a fine filter behind the conventional air filter. Other research group have come with biocide enrichment of the fibrous filtering media for air filters intended for ground transport. As a biocide the chlorhexidine digluconate (CHDG) was used to inactivate the bacteria and viruses. *E. coli*, *Candida albicans*, *St. aureus* methicillin resistant (MRSA) and SARS CoV-2 were successfully eliminated and inactivated in the filter medium of tested air filter (Watson et al. 2022).

The team of air filter developers of the Škoda Auto expressed an interest in the results of bacteriological test provided by our study. Firstly, they wanted to know if any pathogens were detected. Then they asked an independent laboratory to provide a similar study to ours. The laboratory investigated three filters preparing cultivation swabbing the inlet and outlet surfaces of the filters. The results showed mainly *Bacillus cereus*, *Bacillus subtilis* and then *Bacillus sp*. Bacilli were present in inlet and outlet of the filters 1 and 3 and in the inlet surface of the filter 2. Then *Corynebacterium sp., Pseudomonas sp.* and *Staphylococcus sp.* were detected mainly in the outlet surfaces. The laboratory providing the tests did not detect all bacterial species in detail (the list of results is available at the author). The independent laboratory detected similar bacteria as our study, moreover majority of the detected microorganisms are environmentally present. They did not detect any pathogens as well.

7.5. Bus Airconditioning System

A wide range of different bacteria was detected. The bacterial species ranking among the members of genus *Bacillus, Brevibacillus, Peribacillus, Burkholderia or Cytobacillus* are environmentally ubiquitous. They can live in dust soil and air, so their presence on the inlet surface of the air filters in the bus is obvious. Their

ability to produce spores gives them the potential to survive in very unfavorable conditions and their resistance to the effect of environment can preserve their viability on dry air filters for long time. It was suggested that *Bacillus atrophaeus* survived on the surface of HEPA filter for 210 days without any loss of vitality (Mittal et al. 2011). The bacteria from the genus *Bacillus* are relatively large but the results show that they penetrate the filter. *Bacillus cereus* is a large rod-like bacterium – the vegetative cells are 0.5 by 1.2 to 2.5 by 10 μm and occur singly or in chains (Schoeni and Wong 2005). It is considered as a potential pathogen; it is known most frequently as the cause of the food poisoning. The described infections affect the eyes – endophtalmitis (David, Kirkby, and Noble 1994), skin – wound infections, brain – meningoencephalitis (Gaur et al. 2001). These infections typically occur in immunocompromised persons. Nevertheless, *Bacillus cereus* caused meningoencephalitis in a person with healthy immune system (Worapongsatitaya and Pupaibool 2022). *Bacillus licheniformis* serves as natural decomposer, living in the soil and the spores could be present in the dust. *B. licheniformis* is primarily pathogenic for insects, can be used as a component of probiotics but it was reported as a cause of food poisoning as well (Salkinoja-Salonen et al. 1999). Although *B. licheniformis* is considered nonpathogenic for humans, it can be responsible for infections of eyes and recurrent sepsis (Haydushka et al. 2012). On the outlet surface of the filter 4, *Bacillus thuringiensis* was identified. It was also identified as potential pathogen for humans. It can cause pulmonary infections suffering from neutropenia (Ghelardi et al. 2007). Identified bacteria ubiquitous in the ambient air can be in higher concentration in the indoor air of the bus and moreover, the bacteria identified on the outlet surface of the filter may recirculate back to the passenger area of the bus. The urban buses are characteristic with fast passenger exchange because of high frequency of stops with door opening and relatively short time of stay in bus cabin. Most of immunocompetent passengers are not in risk of infection caused by described bacteria. Only persons with naturally decreased immune system function such as elderly people and small infants have to be aware of some risk.

Technically, the $PM_{2.5}$ filters are installed to the HVAC system to protect mainly the evaporator from dust, pollen and other particulate matter contamination and obstruction. The air filters intended for particulate matter filtration perform well. The question which still remains is how to improve the passengers' protection from bioaerosols and airborne pathogens transmission. The economic burden of more frequent filter change or finer filters use is obvious. This topic may be more profound when considering transit and coach buses in

which the HVAC system is set on recirculation inside the passenger's cabin more frequently.

The contamination of both surfaces of the air filters of urban buses was not high. The quantification revealed that the microbial burden is low. When considering the demounting of the air filters in the ambient air, the bacterial contamination can have the origin also in incorrect handling of the filters or omitting the aseptic conditions. Manipulation with the filters without protective gloves may cause contamination of *Staphylococcus epidermidis* and *Staphylococcus warneri*. These bacteria are commensals of human skin and improper manipulation with the filter can cause undesirable contamination. The *Staphylococci* was identified in four filters – on the filter 3 and 4 on inlet and outlet surface comparably. So, we think that the undesirable contamination could be excluded. In the filter 3 and 4 the *Staphylococci* were identified on both surfaces of the filters. Even there, we should take into regard the possibility of recirculation because these bacteria are potential human pathogens. *Staphylococcus epidermidis* can cause various infections of blood stream, endocarditis or wounds (Vuong and Otto 2002). *Staphylococcus warneri* is also potential pathogen and was reported as a urinary tract infection cause. Despite the described cases are mainly the examples of nosocomial infections, the recirculation of these bacteria may seem a problem for sensitive persons. Moreover, both species are capable to produce biofilms which can contaminate the airways of the air conditioning system and enhance the bacteria recirculation (Kanuparthy et al. 2020).

Low bacterial contamination and absence of human pathogens may be caused by the summer season when the filters were removed from the urban buses air conditioning system. In summer season people usually do not suffer from respiratory diseases and usually use the public transport less frequently because of vacations. Even though the urban buses may be sometimes crowded and the concentration of bioaerosols rises, we did not detect any pathogenic bacteria. Absence of pathogens may be caused by several facts – the pathogenic bacteria are sensitive to ultraviolet radiation and dry conditions of the air of summer season. As reported previously, the pathogens can survive on the filter surface for very short time (Mittal et al. 2011). So, we did not detect any viable pathogens. Secondly, the urban buses can have the windows open and the doors open quite frequently. Then, the air circulates faster and the indoor air of the bus cabin is diluted by the fresh air coming from outside. It was reported that the windows opening can significantly lower the bioaerosols concentration (Edwards et al. 2021).

7.6. Experiment with Portable Air Purifier and Nanotextile

Viruses are the biggest problem for air filters. Smaller viruses, which are usually smaller than 300 nm, are not completely eliminated by HEPA filters. Consistent with this claim, the nanotextile monolayer did indeed contain coronavirus 229E. It has been shown previously that HEPA filters do not capture viruses adequately. This may be due to several factors. Firstly, viruses can enter the air filter through defects in the material itself, caused for example by pleating of the individual layers of the filter medium. Pinhole leaks may be another cause of filtration capacity failure (Harstad and Filler 2007). Other studies on HEPA filter efficiency also report limited capture of viral particles by HEPA filters (Helmbuch, Hodge, and Wander 2007). HEPA filters operate at the level of a HEPA filter that filters 99.97% of MMPs. The results of the present study are quite consistent with our previous studies revealing poor virus capture in HEPA filters. In particular, coronavirus 229E penetrated the HEPA filter in our previous study of a home air purifier equipped by HEPA filter (Obitková and Pavlík 2019). Adenovirus is also significantly small, with a diameter in the range of 70 to 100 nm. This suggests that it can penetrate through the filter. The occurrence of this virus on the inlet surface of the filter may be due to the droplet mode of transmission of this virus (Baron 1996). Droplets are removed from filtered air by HEPA filter more efficiently than for example aerosols. The COVID-19 pandemic prompted more rapid development of personal protection measures, including the development of face masks and respirators made of nanomaterials. Nanotextiles are a promising means of air filtration. Our selected monolayer of nanotextiles captured coronavirus 229E. This pathogenic virus is small, suggesting that nanotextiles could be a sufficient means of air filtration. The nanotextile used in this experiment has several key properties. The 50 nm pore size covers the diameter of most human viral pathogens. If the nanotextile served only as a sieve, no viruses of our interest would penetrate the chosen monolayer of nanotextile. On the other hand, the small pores of the nanotextile may also be an obvious disadvantage. Nanotextiles could not be used as the sole filter medium due to clogging of the pores by dust or similar larger particles present in the filtered air. Air filters made of nanomaterials designed as a nanofibrous monolayer with a micro fibrous support can have a significantly higher efficiency in eliminating submicron aerosols (Podgórski, Bałazy, and Gradoń 2006). From this perspective, our chosen nanotextile can significantly increase the filtration efficiency of a standard HEPA filter.

As mentioned above, larger particles are usually a challenge for nanomaterial

air filters due to surface loading. The filtered particles from the circulated air are trapped only on the surface of the nanofiber filter. They do not penetrate deep into the filter medium as in the case of conventional air filters. In our last experiment we encountered surface loading of the nanotextile monolayer after 100 h of filtration (Obitková and Pavlík 2019). Moreover, some viruses have short lifespan in the external environment – adenoviruses can survive on the fabric for less than 24 h, and influenza A and B viruses can survive on selected surfaces for 24-48 h (Pirtle and Beran 1991). Therefore, we decided to replace the nanotextile monolayer three times with a replacement interval of approximately 33 h. From a technical point of view, we are unable to estimate the lifetime of the nanomaterial because no information was provided by the manufacturer of the nanomaterial used in the experiment.

The Respiratory SARS CoV-2 Panel real-time PCR detection kit running on the QIAStat DX® 1.0 platform was a suitable choice to cover most of the respiratory viruses we were primarily looking for. The Respiratory Virus Panel is designed to cover all major causative agents of upper and lower respiratory tract infections. Operation of the analyzer is simple and the results obtained were sufficient for our purpose. The RT PCR technique provides very reliable detection of viral nucleic acid. For future research, the quantification of viral load on the nanotextile monolayer or the viability of the detected viruses could be investigated. Although the minimum infectious dose for virus-induced diseases is very low, we believe that quantification of HEPA filter-transmitted viruses could at least be of interest. Furthermore, the QIAStat SARS CoV-2 panel used did not allow differentiation between different types of adenoviruses. To find a specific type, we would use an adenovirus-specific analysis kit in any type of RT PCR cycler.

The HEPA filter was contaminated with adenovirus. In the spring season, when respiratory infections are more common, a second member of the experimental household may have been the source of the adenovirus. The chosen multiplex RT PCR analyzer does not allow specification of adenovirus, so we cannot accurately determine the type of adenovirus. We can only assume that the detected adenovirus originated from the respiratory tract, as adenoviral conjunctivitis is more typical for the summer season and adenoviral infections of the digestive or urinary tract are spread through urine or stool (Goering et al. 2018). On the other hand, the source of coronavirus 229 E is well known and was found in the nasopharynx of one of the members of the experimental household. This household member may have been in the incubation period of the upper

respiratory tract infection.

The current experimental setup was designed to be easy to install with a small number of samples. In comparison to other studies of portable air purifiers equipped with HEPA filters provided indoors – home or school (Rodríguez et al. 2021) (Myers et al. 2022), our experiment is unique in that swabs of the HEPA filter surface and the nanotextile monolayer demonstrate the presence of viral contamination. In their study, Rodriguez et.al. conducted a study on the effectiveness of portable air purifiers in eliminating SARS-CoV-2 in several households in different cities and demonstrated an 80% elimination efficiency of air purifiers. Lindsay et al. also simulated the effectiveness of an air purifier in a single room (Lindsley et al. 2021). We are aware that the experiment from one household could not be generalized to objective information. Contamination of the HEPA filter or the monolayer of nanotextiles could have occurred during the replacement of the nanotextile or during laboratory procedures on both the HEPA filter and the nanomaterial. Despite the simple experimental setup and small sample size, we have provided valuable data in investigating the ability of nanomaterials to trap viruses and increase the efficiency of conventional air filters.

7.7. Nanomaterials

In our experiments, the nanotextile made of electrospun polyamide 6 showed good results in coronavirus interception. Other nanomaterials were tested to confirm their capability to capture fine particles including microorganisms, especially viruses. Some materials could be enriched by inorganic salts or oxides which can inactivate the microorganisms. The polyacrylonitrle (PAN) nanomaterial encapsulated with titanium dioxide (TiO_2) shows good filtration capacity for particles in field tests and in laboratory test against laboratory generated ammonium sulphate particles of 10-700 nm. Moreover, the titanium dioxide has a photocatalytic effect which helps to degrade inorganic compounds present in particulate matter. The TiO_2 was also shown to have potential in influenza virus inactivation (Goel et al. 2022). Polyacrylonitrile belongs to the polymers which are together with polyamide 6 (PA6) and other similar synthetic polymers to the group of materials having the best characteristics for electrospinning. Electrospun polyamide has superior fiber forming ability, is efficient in particulate matter elimination, can capture particles sized in nanometers. Another advantages of PA fibers are represented by biodegradability, humidity and water resistance. These characteristics are optimal

for nano fiber-based air filters fabrication. Some studies made on the PA6 derivative PA6/6 revealed that PA6/6 has selected parameters better than PA6. Nevertheless, PA with its subtypes is a particularly attractive material for filtration applications (Matulevicius et al. 2014).

The use of nanofibrous membranes can help to reduce airborne particulate matter because of their large surface area, extremely porous structure, and adjustable pore size. However, despite their unique properties, the main drawbacks of nano fiber membranes are their poor mechanical properties. In our experiments the PA 6 nanotextile was fixed to the nonwoven polyester base in order to prevent mechanical injury of the nano material. Currently majority of nanofiber-based filters need to be placed on some supporting medium.

Poor mechanical characteristics are related to microplastic production. A proportion of PM consists of microplastics (MPs), defined as fine plastic particles that are less than 5 mm in size. As PM, MPs can induce various toxicities in organisms and have therefore attracted increasing attention since their first occurrence was detected in the aquatic environment (Thompson et al. 2004). Based on their structural characteristics, microplastics can be categorized as fibers, foams, pellets, films, and fragments (Yadav et al. 2023). It has been found that air conditioning systems, which are widely used to regulate room temperature and recirculate mostly indoor air, can act as both a sink and a source of FMPs (Chen et al. 2022). The nanomaterials are widely used in household workers protection and protective equipment in healthcare, where mainly face masks are produced with nanotextile application. The nanotextiles have excellent characteristics in viruses capture therefore the face masks covered or made of nanotextiles were successful (El-Atab, Mishra, and Hussain 2021). Disposable plastic face masks consist of two outer layers of microfibres and an inner layer of nanofibres. It was reported that one piece of the mask can release up to $1.6–3.8 \times 10^9$ nanoplastics (size < 1 μm) and $1.3–4.4 \times 10^3$ microplastics (size 1–600 μm) (Ma et al. 2021).

The electrospinning technique allows the design of nanofibre diameters in a wide range from 40 to 2000 nm using a suitable combination of polymers and solvents. Electrospun nanofiber filters can have excellent properties, such as high surface-to-volume ratio, controllable morphology and connectivity, and low-pressure drop. These characteristics make them attractive for achieving excellent $PM_{2.5}$ filtration performance. Electrospinning may seem a complex process depending on many parameters but the result has many significant advantages – electrospinning produces 3D nanofibres; it allows the production of thin fibres with small diameters down to tens of nanometers; it allows the processing of a

wide variety of syn-thetic and natural polymers, even in combination, or containing nanometer-sized inorganic particles; it is cost-effective compared to fiber extruders operating at high temperatures, and has the potential for scale-up production (De Riccardis 2023).

Recently, the environmental and economic sustainability attracts the attention to biodegradable and environmentally friendly materials. They are usually processed by electrospinning and can have good efficiency in particulate matter elimination. Different natural materials are tested to produce bio-based polymers. Production of bio-polyester was reported. However, several disadvantages have appeared during the processing of polymers – toxic solvents as toluene, which are released from the material (Cho et al. 2020).

Both synthetic nanofibrous filters and bio-fibers have some excellent properties. It depends on purpose of use which of them can be chosen and there is a wide area for investigation of the best properties in microorganism elimination.

8. 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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