

Testing an innovative device against microbiological airborne contamination

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Abstract

Aspergillus fumigatus is a major airborne nosocomial pathogen in immunodeficient patients. We evaluated the efficacy of an innovative mobile air treatment device against this resistant pathogen.

A flash and massive artificial air contamination (10^7 conidia of *A. fumigatus*) was conducted in a high security level room (L3). Sequenced air samples were performed to measure the airborne biological effect of the device.

Once the high contamination level being obtained, the concentration decay of conidia in the air was reduced of 95% in 18 minutes and its quasi-complete destruction (99%) was obtained in 29 minutes, even at the lowest output of air flow during the operation of the machine.

Comparatively, during spontaneous decantation, conidia remained airborne for over 1 hour (241 UFC/200 L). No toxic molecule (volatile organic compounds and ozone) was released by the machine when operating in standard conditions.

This indoor air contamination model provides consistent and reproducible results. The air purifier proved efficient against a major contamination, even at low airflows when comparing the different decontamination kinetics; it is safe to be used in human presence. Its use should be recommended to prevent air transmitted diseases.

Introduction

The incidence of nosocomial fungal infections keeps on dramatically increasing (1). Airborne contamination represents a major risk for nosocomial infections, especially to immunocompromised patients (2). Many airborne microorganisms become opportunistic pathogens, which can especially affect neutropenic patients (3) or patients undergoing corticoid therapy (4). For instance, molds like *Aspergillus* are widespread fungi that are particularly virulent in this context (5), especially the species belonging to the section *Fumigati* (6) (7). Invasive aspergillosis is responsible for high mortality rates (2). The cost and side effects of the effective antifungal drugs can limit their use (8).

Some epidemiological studies have clearly demonstrated an epidemiological relationship between the presence of *Aspergillus* in the air and the outbreak of nosocomial aspergillosis (9) even when immunodeficiency is not so heavy (10).

One of the most relevant ways to prevent the spread of these airborne pathogens is to avoid one's exposition to environmental *Aspergillus* conidia. High-risk patients are usually isolated during aplasia, in a specific room that provides a near-sterile environment and helps prevent airborne transmission of pathogens (11). The systematic filtration treatment of distributed air is meant to retain any pathogenic airborne microorganism, thereby providing safe environment. Moreover, air quality is controlled by conducting systematic air sampling in these types of rooms (12).

Actually, the primary concern of most hospitals is to improve air quality in an effort to reduce the risks of infections, particularly the ones caused by *Aspergillus*' spores (13) (14) (15).

Different technological processes can be used for air treatment (*e.g.*, HEPA (High Efficiency Particulate Air) filtration, regulation of air pressure) (16) (17), but new and innovative technology is being developed and expected to improve this trend (18).

Filtration is no longer the golden standard for air treatment. It is known to be energy consuming and may stock and release pollutants. It may also allow the growth of microbiological agents on the filter itself (19). Air treatment devices should destroy airborne pollutants without generating toxic compounds, especially if they are meant to be used in the presence of humans.

AirLyse technology is designed to eliminate microbiological airborne contaminants. It combines inactivation by physical stress (crash on a rigid support and UVC germicidal effect), with final destruction of residues by photocatalysis (self-cleaning). The AirLyse air purifier is a mobile standalone device (“plug and play” system) that can be easily used in healthcare facilities (e.g., hospital standard rooms). To test the performance of the AirLyse air purifier, we conducted experiments in a closed environment highly contaminated with *Aspergillus* spores. We established an experimental model of aerosolization chamber that allowed assessing the performance of AirLyse by measuring the decrease in particulate and fungal concentrations overtime.

Material and Methods

Microbiological Materials and Methods

Experimental model of Aspergillus contamination and decontamination

Decontamination tests are performed in a 60 m³ (5 x 4 x 3 m) high security (L3 safety level) room (Fig. 1). Walls are smooth to avoid particle retention and facilitate cleaning. The 60 m³ volume corresponds to a standard hospitalization room size. HVAC (heating, ventilation, and air conditioning) system allowed in-coming and out-coming air to be fully HEPA filtrated when needed. Operators were completely protected by wearing a suit and a respirator device against microbiological agents exposition.

The ventilation could be stopped during the experimentation so that no airflows were generated and the room air was totally calm. Air was exchanged between inside and outside on demand (air room washing between two aerosolizations). The temperature and the hygrometry were checked throughout the testing. 10⁷ conidia of *A. fumigatus* were aerosolized in one step through a central commercial jet nebulizer (Atomisor+/NL9M[®], associated to an Abox+ compressor, La Diffusion Technique Française, Saint-Etienne, France) placed at 1.60m above ground level at the center of the room (20). Four mobile stand fans were placed inside the room, on a circle of radius 140 cm and of center materialized by the nebulizer. The fans were started right during the nebulization and prolonged during 4 minutes in order to obtain homogenization of airborne particles throughout the whole room. Sequential air samples were repeated in order to establish kinetic data (T0 just after nebulisation and homogeneization period of time, T5, T10, T15, T20, T30, T45, and T60 minutes).

Inoculum preparation

A homemade clinical *A. fumigatus* strain (reference strain of the mycology laboratory) is first grown on Sabouraud agar at 37°C (Sabouraud GM+C, Becton Dickinson™, France). After 5 days of mycological culture, conidia are harvested. The Petri dish is flooded with a 15mL sterile solution of 0.05% Triton X100® (ICN Biomedicals™, USA) in 0.9% phosphate buffered saline (PBS BioMerieux™, France). Conidia are collected and a first centrifugation (1,700 x g, 10 minutes) is performed. The pellet is recovered in PBS and the spores-washing challenge is repeated once. The spore concentration in this PBS parent-dilution is assessed in a Malassez counting chamber (Kova Slide 10, Glasstic™, USA). The final concentration is *in fine* adjusted at 10⁷ conidia / mL (21)(22).

One millilitre of the suspension of 10⁷/mL conidia of *A. fumigatus* was fully aerosolized. After nebulization experimentation, an aliquot of the remaining suspension is cultured on Sabouraud agar to check up on spore viability and quantity.

Air sampling

Four bio-impactors (MK2 Sampl'AIR, AES Chemunex, France) were placed near the nebulizer, each 1.40 m away from the *Aspergillus* conidia source and at 1.20 m above the floor (average height where air is generally inhaled). Collect of microorganisms is made by air aspiration, through a grid which is situated a few millimeters above a Petri dish (Malt agar plates: 25g malt extract, 20g agar, 1L water). Bioimpactors were turned on simultaneously with a remote control at the start of every sampling test. Repeated samples of 200 L air (100 L *per* minute during 2 minutes) were collected (11), with the air purifier off (at T0) and with the air purifier on. During the first 20 minutes of the experiment, airborne particles were aspirated through the sample device every 5 minutes (T5, T10, T15, and T20). Then air samples were repeated at T30, T45, and T60. One hour after nebulization, stand fans were restarted for 5 minutes to redistribute the possible settled spores. After mobilization, new air samples were taken (T75).

While the samples were being collected, instantaneous particle counting was processed in the air, using channels of different sizes on a LightSource meter.

Air quality analysis

Microbiological samples were analyzed in the Parasitology - Mycology Department of the University Hospital of Tours. After a 48h-incubation period at 37°C in a steam room, the number “*n*” of CFU (Colony Forming Units) of *A. fumigatus* which have grown in the impacted points were counted in each Petri dish (microscopic control). The concentrations of the airborne conidia were expressed per 200 L of air (CFU / 200 L). A maximum of $n=258$ colonies can be counted, depending on the number of grid holes number. Numbers of counted *Aspergillus* colonies are adjusted according to abacuses. In order to take into account the probability in having coinciding impactions of 2 or more conidia, an algorithm is applied (AES, Sampl'air MK2, user's manual). A table indicates the *N* corrected value (probable number of viable impacted particles) corresponding to the *n* value (number of CFU actually numerated). If measurements were concordant, an average was calculated for four malt agar plates sampled concomitantly (*N* means). These data were then entered into a computerized database.

Statistical analysis

Concordance between the results for the four bioimpactors distributed through the room was calculated using the respective *N* CFU falls in different airflow working conditions for the air purifier at each sampling time. The intraclass correlation coefficient for the simultaneous 4 samples was calculated at each airflow value. This coefficient is supposed to be near 1 if the four bioimpactors show similar results.

Modelling the exponential decays of the *N* means values (CFU / 200 L) was performed for each level of airflow. These decreases could be each expressed by the following differential

equation, where λ (lambda) is a positive number called the decay constant: $N(t) = N_0 e^{-\lambda t}$. Each coefficient λ for each airflow level was then compared to the one of the control experimentation (spontaneous decantation), using a Student's t-test and testing the statistical significance by calculation of the p-value. Student's t-tests were also used to compare the coefficients of the 3 airflow levels. The time it takes for a certain percentage of the conidia to decay (decay rate) was calculated using the differential model.

Chemical Materials and Methods

A protocol was designed to evaluate the impact of the AirLyse machine on indoor air volatile organic compounds (VOCs) and ozone (O₃) profiles. Continuous real-time chemical monitoring of O₃ and VOCs was carried out overnight in a standard laboratory room, when no interference caused by human activity could disturb the air composition. The air purifier was on, (airflow 500m³/h) during the first half of the night, and automatically shut down after several hours. The heating, ventilating, and air conditioning (HVAC) systems were maintained constant during air collection.

HS-PTRMS (High Sensitivity Proton Transfer Reaction Mass Spectrometer, Ionicon Analytic) was used as an analytical tool that allows for real-time, quantitative and highly sensitive monitoring of VOCs in air (23). The detection threshold is about a few parts per trillion by volume (pptv) and an analytical precision of about 20%. The PTRMS was placed centrally in the room, near the air purifier and selected VOCs (including a proxy for formaldehyde, acetaldehyde, methanol, acetone, benzene and toluene) were measured continuously. Instrumental blanks were performed automatically (passage through a catalyst removing all ambient VOCs) and subtracted from the atmospheric signal. The instrument was calibrated in laboratory by measuring a standard gas containing the compounds of interest.

O₃ was measured every minute at the air purifier output (upper) with a UV analyser (Model 49C, Thermo-Electron, Franklin, MA).

Air Purifier, AL-PR004, AIRLYSE.

One semi-industrial prototype of an air purifier device was developed in collaboration within our research team and could be freely tested. This patented mobile device is designed for a continuous decontamination of indoor air in presence of humans and thus preventing air transmission of pathogens as it is required (18). Three processes well-known to be lethal for microorganisms are combined on a single media. Airborne particles are crashed on a rigid 3D (3 dimensions) non-occlusive media, generating a physical stress. This media is self-cleaning because of its heterogeneous photocatalytic properties (24). Airborne particles are simultaneously exposed to germicidal UVC radiations through a length-oriented reactor allowing a prolonged exposition. Heterogeneous photocatalysis has greater efficiency when being combined with physical operations (25). The present device doesn't have a retention effect like filter but allows the destruction of the pollutants. Several air flows are available: 0 m³ per hour (machine turned OFF to allow spontaneous decantation), 500 m³ / h, 600 m³ / h and 1000 m³ / h (machine turned ON).

Heterogeneous photocatalysis is an advanced oxidation process; this recent technology has been shown to be a highly promising for environmental purification (26). It is considered as useful for many applications in cleaning water, air and surfaces by eliminating microorganisms, odors, and organic pollutants such as pesticides and combustion compounds (27) (28). It is capable of killing a wide range of Gram-negative and Gram-positive bacteria, filamentous and unicellular fungi, algae, protozoa, mammalian viruses and bacteriophage. Spores are generally more resistant than the vegetative forms, possibly due to the increased cell wall thickness (29) (30). By generating highly oxidizing species (O₂-anion radical and

OH- radical) at the surface of a media coated with titanium dioxide (TiO₂) when being irradiated with UV light, it causes complete mineralization (producing carbon dioxide, mineral acid, and water). TiO₂ is the best-suited photocatalyst, in terms of energetics, stability and non-toxicity and it can continuously exert antimicrobial effects when illuminated. Only ultraviolet (UV) light irradiation can excite TiO₂. A valid alternative to the use of TiO₂ has not been up to now found (31). However, it can occur in some specific conditions, some intermediate may be produced such as VOCs, and especially formaldehyde, which is also a common pollutant of indoor air.

Fungi and *Aspergillus* can be inactivated by UVC radiations under certain conditions (32).

When using germicidal ultra-violet lamps, ozone can be produced in the area and be toxic. It has been determined that O₃ exposure has a negative impact on human health (33). The “Low ozone” lamps fitted to AirLyse transmit up to 90% of their energy at the 254nm wavelength (typically utilize a doped fused quartz that blocks the emission of 185nm energy) and avoid ozone generating.

Results

Microbiological Results

A total of 144 air samples were analyzed, with 36 samples for each of these 3 conditions: spontaneous decreasing test with 0 m³/h, followed by air treatment at 500 m³/h, 600 m³/h and 1000 m³/h. These conditions correspond to 4 replicates (4 samplers distributed around the room) in 9 time points of measurement at T0, T5, T10, T15, T20, T30, T45, T60 and T75 minutes. For every experimental condition, *n* CFU / 200 L were observed (data not shown). The 4 distributed bioimpactors showed very similar results when sampling at the same time during all experimentations. High values of intraclass correlation coefficients showed an important concordance between the four agar dishes, for the same time of sampling (Table I).

Immediately after nebulization, all of the air samples were saturated with *Aspergillus* conidia (T0 malt plates were totally invaded by fungal colonies, $n > 258$ colonies).

Spontaneous decrease (AirLyse air purifier turned off) was a linear spontaneous decrease of *Aspergillus* spores : 57% % at 15 minutes, 42 % at 30 minutes and remained detectable for 1 hour after aerosolization (22.8% of initial inoculums) (Figure 2).

On the contrary, we found that a complete and fast purge of the saturated atmosphere occurred when the purifier was operating. One log₁₀ of conidia decrease was obtained in 7 minutes for 500 m³/h. 2 log₁₀ of conidia decrease was obtained in 14 minutes for 500 m³/h. 5 log₁₀ of conidia decrease was obtained in 34 minutes for 500 m³/h.

To compare to the level at T0 (maximum contamination), 95% of decontamination was obtained in 18 minutes at 500 m³/h, 18 minutes at 600 m³/h and less than 16 minutes at 1000 m³/h. Whatever the air flows level of AirLyse air purifier (500 to 1000 m³/h), 99.99% of conidia was eliminated in less than half and hour (Figure 2).

The λ value was determined for each decontamination test: for 1000 m³/h, $\lambda = 0.141$ [0.122 - 0.159]; R²=98.1%); for 600 m³/h, $\lambda = 0.153$ [0.133 - 0.173]; R²=98.0%); for 500 m³/h, $\lambda = 0.147$ [0.131 - 0.164]; R²=98.5%). For the spontaneous decreasing test, $\lambda = 0,023$ [0,018; 0,028], R² = 94.3%. The decontamination curves ($N(t) = N_0 e^{-\lambda t}$) are represented in figure 2.

No statistical difference was found between the λ values when we compared the three different air flow levels with each other's. That means that the decontamination kinetic could not be considered as different at the three different air flow levels, even at the lowest airflow level (500 m³/h).

Conversely, the λ value was statistically different when comparing the one value for spontaneous decreasing test to the other tests when the air purifier was turned ON ($p < 0.001$ for each of the three comparison tests).

The measurements for particles counting are shown in figure 3. The decrease in particles $> 1 \mu\text{m}$ was clearly boosted when AirLyse was on, whereas the decrease was much slower when the unit was off.

Chemical Results

Continuous VOC and ozone profiles do not significantly change when the mode of operating is modified (air purifier switched off after several hours of operation, experience repeated twice). These profiles were the same when focusing on any of the measured VOCs, especially formaldehyde. Interestingly, we noticed that an unexpected outdoor traffic-related source modified the indoor VOC profile by detecting the presence of combustion compounds (weekly nitrogen delivery by truck), confirming the sensitivity of the measurement method.

Discussion

Multidisciplinary collaboration in research about air quality is a huge issue. Having access to high-technology equipment and environment is crucial because chemical and biological processes have to be properly interpreted. Implementing methodologies and interpreting findings requires highly competent teams. New models have to be created for evaluating indoor air quality.

As long as toxic molecules are in low concentrations in the air and can thus generate chronic intoxication, the selected method of detection should allow for a very low detection limit.

A. fumigatus is a highly resistant air contaminant and can cause lethal damage to several organs when developing in an immunodeficient organism. Its eradication in the near

environment of fragile patients is essential (34). Experimental models with microbiological consistency have to be set up in order to better control dissemination and how to capture and destroy the conidia. Air purifiers have to demonstrate scientific efficiency and safety.

Even when using a highly sensitive detection method, no release of VOC or O₃ was detected in the room when the air purifier was on compared to off. Even if biological detection tools are known to be with poor reliability in the air, the experimental model allowed validating the design and the biological results.

This study encourages pursuing the work of improving indoor air quality study models. A better understanding of the spontaneous behaviour of microorganisms in the air should help improve the design of prevention procedures against air-transmitted diseases. A thorough knowledge in chemical and biological phenomena should be a defining element for implementing safety in prevention procedures.

The control of airborne microorganisms represents a major issue in hospitals and healthcare facilities (35). Despite their rare occurrence, outbreaks of invasive aspergillosis remain problematic due to high mortality rates (2) (36). A few processes have already been performed to decrease the risk of onset (37) (38) (39), such as high pressure rooms or HEPA filters (16) (17), or the use of bio cleaning surfaces (40).

This study describes a challenging experiment to assess the destruction of *Aspergillus fumigatus* conidia, using an innovative mobile air purifier. The model of fungal contamination presented here appears relevant. Conditions of aerosolization, homogenization, and sampling are standardisable. The chosen volume of the experimental room is 60 m³, which is comparable to the size of a hospitalization room. The method of aerosolization presented here seems satisfactory, in spite of a saturation of the atmosphere due to the high fungal burden. We chose to place the four bioimpactors at 1.20 m above ground level because it represents

the average height of a person whose position varies between sitting, lying down and standing up. It is important to note that the concordance is almost perfect between all 4 agar dishes, at every sampling time, which demonstrates the reliability of the 4 bioimpactors and, consequently, of the measurements. Nevertheless, it would be interesting to repeat these experimental tests in additional series to further establish our results. We can also note that there was no other fungal species detectable on the Petri dishes, which proves that there was no interference with pre-existing airborne environmental fungi in our model of contamination. One of the limitations in our model is the fact that the room was closed during the experiment. In hospital rooms, it is common to have opening and closing of doors, due to entry and exit of medical and nursing staff to provide care, deliver meals, clean, etc.... Indeed, indoor concentration of molds is not only influenced by indoor activities but also by movements on the outside. It is an important consideration because *Aspergillus spp.* can persist in a hospital environment for long periods of time (2) (41).

In extreme conditions of saturated atmosphere, we can presume that the AirLyse technology, combining three germicidal processes on a single media, is effective and leads to destruction of the conidia. In controlled conditions, when the machine is OFF, the concentration of particles in air, resulting from *A. fumigatus* nebulisation, remained high during the entire experiment. As a whole, our experiment showed that the AirLyse air purifier has the capacity to decrease the fungal load in atmosphere, even in challenging conditions. It is important to note that lowering the air flow is not compromising the efficiency of the AirLyse air purifier. Low air flow output is generating less turbulence and consequently emitting less noise, and allows taking into account the comfort of the patient and health workers.

These results indicate that the air purifier could be used to protect immunocompromised patients, and, on a larger scale, to control indoor air quality in any area prone to high levels of particle contamination. Obviously, this unit is not intended as a replacement for manual

cleaning and disinfection. We can rather consider it as a complementary method in standard prevention measures to reduce risks (mold source eradication, meticulous room maintenance, and environmental surveillance) (42) (40). In hospital environments, the AirLyse air purifier could also represent a temporary solution for patients at risk, who are placed outside of standard protected areas. We can also imagine that this device could be useful as an addition to or in combination with other treatment processes during remodelling, or renovation or construction projects (43). It is proven that procedures aiming to reduce airborne conidia burden are associated with a decrease in the occurrence of invasive aspergillosis (9) (34). Finally, it should be relevant to perform a prospective clinical trial about AirLyse air purifier, even if invasive aspergillosis is too rare to establish the real benefits of the system on mycotic infections occurrence. Airborne decontamination should be the right environmental surrogate market.

There is a number of mobile air-treatment units that have been described (44), and here, we focus on the characteristics and efficiency of a destruction processes-combined technology. We can however imagine that other tests could be performed with different *A. fumigatus* inoculum sizes or other environmental microorganisms like miscellaneous *Aspergillus sp.*, *Staphylococcus aureus*, influenza virus.

Conclusions

Multidisciplinary teams usually collaborate on indoor air quality work, from science to concrete prevention: chemical, microbiological, medical and industrial. Real-size models are required to better understand the successive steps of aerosolization, distribution, survival, dissemination, captation and inactivation of airborne pathogen agents. *A. fumigatus* is not the sole responsible microorganism for air-transmitted diseases; it would be useful to conduct studies on other fungi (*Scedosporium*, *Fusarium*, *Pneumocystis...*), bacteria (tuberculosis,

pneumococci, multi drug resistant bacteria) and emerging respiratory viruses, which are the challenge of the current decade. Tests should not be different regarding the type of germs tested when considering the non-specificity of the germicidal processes that are combined in the Airlyse air purifier. But some airborne conditions and some external resistance parameters can generate some specificity regarding the pathogen to be trapped and killed.

Collaboration should happen very early when developing devices for the healthcare industry.

This would provide access to scientific evaluations and ensure that the proper technology will address the current issues with accurately and safely.

To conclude, it should be possible to use the AirLyse prototype, not only for specific medical purposes, but also as a tool for the pharmaceutical industry, and even for food-processing companies...

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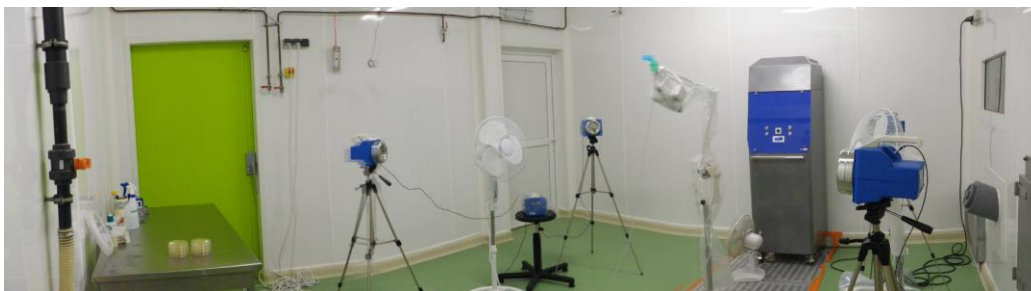
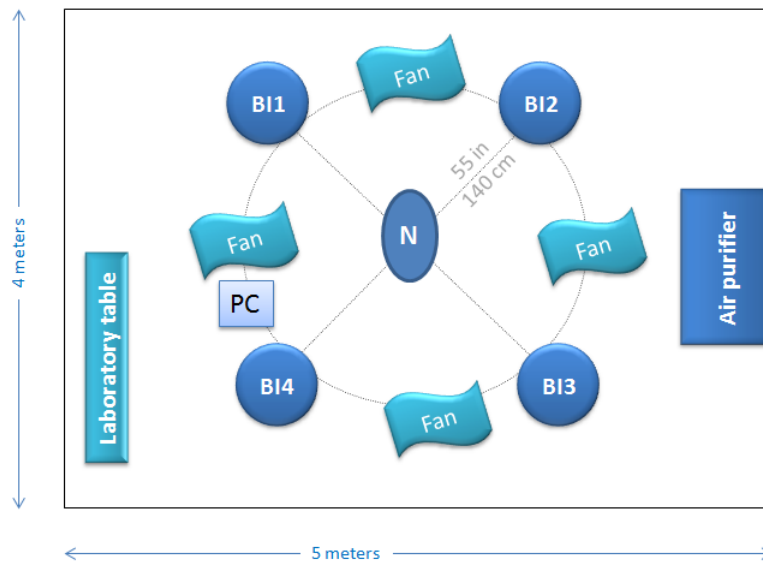
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Table I Intraclass correlation coefficients expressed for every air-flow output functioning.

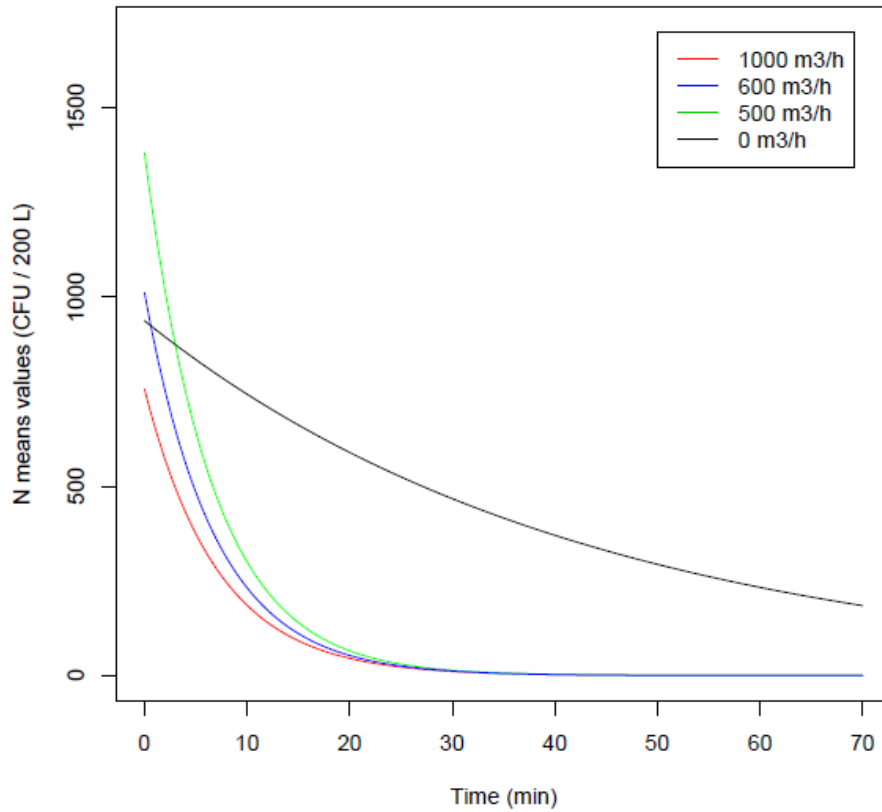
treatment procedure speed of flow	intraclass correlation coefficient [range]	number of samples
0 m ³ /h (spontaneous decrease)	0.927 [0.826 ; 0.979]	36
500 m ³ /h	0.957 [0.895 ; 0.988]	36
600 m ³ /h	0.997 [0.993 ; 0.999]	36
1000 m ³ /h	0.947 [0.871 ; 0.985]	36

Figure 1: Experimental room for massive *Aspergillus* air contamination



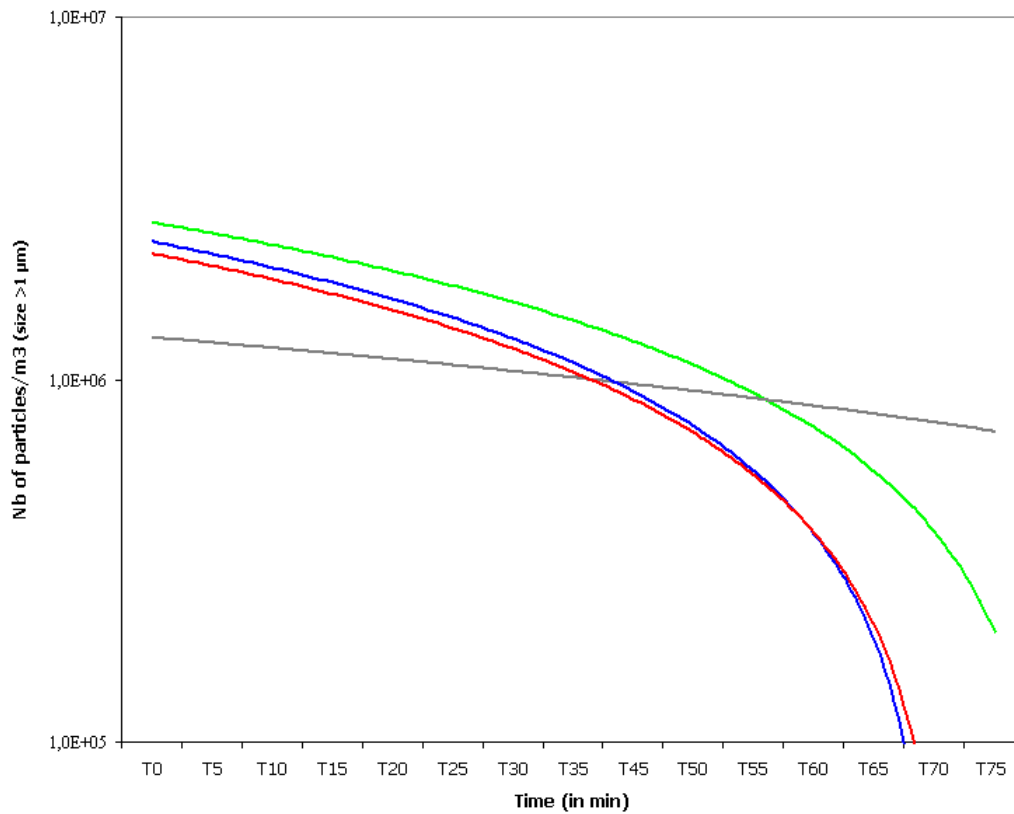
Map and picture of the aerosolization room at biosafety level-3 facilities. Experimental procedure requires a central nebulization equipment (N), a mobile air-treatment unit (AirLyse air purifier), 4 bioimpactors (BI) for air sampling, 1 particles counter (PC), 4 Fans (Fan). Operator'protecting suit and respirator device.

Figure 2: AirLyse purifier effect against massive *Aspergillus* air contamination under various airflows conditions.



Modeling of n means (UFC / 200 L) for every flow speed of eparation, with $n(t) = n_0 e^{-\lambda t}$ (red curve: in conditions of AirLyse purifier is ON at 1000 m³/h; blue curve: with AirLyse purifier ON at 600 m³/h; green curve: with AirLyse purifier ON at 500 m³/h; grey curve: in conditions of spontaneous decrease with AirLyse purifier is OFF at 0 m³/h).

Figure 3: Air particle counting during massive *Aspergillus* contamination under various airflows conditions.



Number of particles $> 1 \mu\text{m}$ counting with the LightSource® meter (red curve: in conditions of AirLyse purifier is ON at $1000 \text{ m}^3/\text{h}$; blue curve: with AirLyse purifier ON at $600 \text{ m}^3/\text{h}$; green curve: with AirLyse purifier ON at $500 \text{ m}^3/\text{h}$; grey curve: in conditions of spontaneous decrease with AirLyse purifier is OFF at $0 \text{ m}^3/\text{h}$).