Air quality monitoring is essential when it comes to protecting the urban population, especially that of big metropolises, from biohazards including biopathogens (BPs). This process is aided by different samplers and analyzers of aerosol pollutants, filters and disinfection systems. Their performance is tested using experimental aerosol formulations with a predetermined composition. Unfortunately, the majority of such systems available in Russia are only able to process a few hundred liters of air per time unit, which is too little. Big aerosol chambers (10 to 20 m³) are very expensive and may not fit into a lab, necessitating an extensive overhaul. In this work we present a workstation for the detection of BP markers under controlled conditions based on the microbiological safety box MSB-III-Laminar-C-1.5 (380.150.01) that was originally designed to test the performance of samplers and analyzers of experimental aerosol formulations. Our workstation can handle the majority of BSL-1-2 BPs and, given the chamber volume that satisfies the requirements of aerosol experiments (> 4 m³), can be installed in a lab with an area of > 10 m².

Keywords: infection, aerosol, aerosol sampling, aerosol chamber

Funding: this work was supported by the Ministry of Health of the Russian Federation as part of the project The National System for Chemical and Biological Security of the Russian Federation (2015-2020) and by the Ministry of Education and Science as part of the project RFMEFI60117X0018.

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Received: 08.06.2018 Accepted: 31.08.2018
DOI: 10.24075/brsmu.2018.053
the ground for the spread of airborne infectious aerosols [2]. In addition, bioterrorism still poses a serious threat [3, 4].

Fortunately, we have an extensive arsenal of tools for monitoring air contamination at our disposal capable of sampling aerosol particles, estimating their concentration and size, and identifying biopathogens contained in the sample. Obviously, adaptation of these devices to a specific task and improvement of their performance are impossible without proper testing involving the use of experimental aerosol formulations that model the behavior of the actual particulate matter. In an enclosed indoor aerosol test chamber, protection from hazardous bioaerosols can be ensured by state-of-the-art high-performance filters [5, 6].

Among the most important parameters the chamber has to meet are sufficient space to accommodate the equipment; easy access inside; reasonable dimensions compatible with the size of the room it will be installed in; the inbuilt system of incoming and exiting air filtration; even distribution of aerosol particles throughout the chamber volume; simplicity of cleaning and decontamination procedures. Modern class III biological safety cabinets (BSC-III) meet the majority of these requirements but one for the inner volume of the chamber [6].

I. Design of the experiment

Our bioaerosol test chamber devised for the detection of biopathogens in the controlled environment is a modernized version of the BSC-III Laminar-C-1.5 (380.150.01) designed to test samplers and analyzers of model bioaerosols.

A prototype of an aerosol sampler with liquid phase recirculation and preconcentration of particles was developed at the National Research Nuclear University MEPhI. The sampler can operate at a volume of air flow of 4,000 l/min collecting particles of >0.5 µm in size. Its performance was compared to that of the SASS 4000/2300 aerosol concentration device with a cyclone air sampler (Research International Inc.; USA).

The main stages of chamber development and testing procedures are described below.

II. Preparation

1. Equipment

1.1. Chamber for the detection of hazardous bioaerosols in the controlled environment

BSC-III Laminar-C-1.5 (380.150.01) by ZAO Laminar Systems, Russia, is a class III biological safety enclosure (GOST R EN 12469-2010) with chamber dimensions of 1.5 × 1.5 × 1.9 m (Fig. 1). Before entering the chamber, the continuously supplied air passes through a high-efficiency class H-14 inlet HEPA filter. The filter maintains a clean environment in the chamber corresponding to ISO class 5 of air cleanliness for 0.5 µm particles (GOST 14644-1-2002). The air exits through a cascade of 2 consecutive class H14 exhaust HEPA filters. In the chamber, the negative pressure of at least 250 Pa (relative to the outside pressure) is created by exhaust fans, forcing the air to circulate in the chamber. The maximum air flow rate through the chamber is 800 m³/h but it can be reduced or terminated by adjusting the rotation speed of the exhaust fans and the position of a gate valve that changes the aerodynamic drag in the inlet air duct. The air containing aerosol particles generated in the chamber goes through a cascade of exhaust filters for decontamination and exits into the room where the workstation is installed. The negative pressure of 250 Pa maintained in the chamber minimizes the risk of aerosol leakage, which depends solely on the efficiency of HEPA filters cascade. This chamber is not equipped with separate air pipes for the aerosol-contaminated air, so the exiting air just travels straight into the first exhaust HEPA filter.

The equipment that needs to be tested is loaded into the chamber through a full-sized leak-tight door. The biaxial hinge mechanism and 4 handles allow the glass surface of the door to fit tightly against the door seal.

Inside the chamber, the environment is controlled by the monitoring equipment connected to a feedthrough panel. Another panel has all necessary airpipe fittings and electrical connectors to connect diagnostic and measuring equipment to the computer.

The operating mode is selected using a control sensor panel. The system can automatically maintain a preset air flow rate and negative pressure in the chamber and signal any abnormal situation or equipment failure. It also reports clogged HEPA- and prefilters and adjusts the fan speed and the position of the gate valve to compensate for a change in their aerodynamic drag.

Disinfection is performed according to standard protocols considering the specifics of the conducted tests. The chamber is equipped with a pair of UV lamps for primary disinfection. Terminal decontamination can be performed using formaldehyde or hydrogen peroxide vapor phases. The equipment required for decontamination can be plugged into 38-mm feedthroughs.

1.2. Aerosol sampling device (National Research Nuclear University MEPhI; Russia)

The aerosol sampler used for our tests consists of a virtual impacter connected to a cyclone by an air pipe (Fig. 2). The device exploits the idea of two-stage particle concentration, allowing to achieve high volumetric air flow rates of up to 4,000 l/min. First, air is sucked into the nozzle of the virtual impacter where captured particles are concentrated. Then the air containing concentrated particles exits the impacter at 300 l/min and enters the cyclone collector where the captured particles precipitate in the sorption liquid circulating in the cyclone. The longer the circulation time, the higher the concentration of the incoming particles in the liquid phase. The volume of the released liquid sample is 7–8 ml.

1.3 Air sampling device SASS 4000/2300 (Research International Inc.; USA)

The air sampler SASS 4000/2300 utilizes a similar principle of action. In the first stage, the air flows into the impacter nozzle at 3,600 l/min. In the second stage, the cyclone collector ensures the flow rate of 325 l/min and releases 4–5 ml of the liquid sample.

1.4 Portable aerosol particle counter SOLAIR 3100 (Lighthouse Worldwide Solutions; USA)

This device is used to test the integrity of inlet and exhaust HEPA filters. In our experiment, we took measurements using 6 particle channels: 0.3, 0.5, 1.0, 3.0, 5.0, and 10.0 µm.

1.5. Aerosol counter of submicron particles 4705 (AeroNanoTech; Russia)

This portable aerosol counter of submicron particles is intended for measuring the concentration of particles and their size.
distribution in the submicronic range. Five particle channels (1, 2, 3, 5, and 10 μm) were used in our experiment.

III. Tests

1. Integrity of HEPA filters (Test 1)

The integrity of the filters was checked before the chamber was put into operation, as recommended by GOST R ISO 14644-3-2007 [7]. The filters were challenged with a control aerosol formulation. Filter surfaces and holding frames were scanned using the SOLAIR 3100 particle counter; alternatively, particle count was performed in the samples of the filtered air collected from the air pipe.

2. Rate and efficiency of air decontamination (Test 2).

Efficiency test of the aerosol sampler designed at the National Research Nuclear University MEPhI

Decontamination tests were conducted in 3 different operating modes. Before the tests, the fans installed in the chamber to set the air in motion and to ensure even distribution of aerosol particles throughout the chamber volume (Fig. 1) and the filtration system were switched on. Upon reaching the air cleanliness of 0–353 particles per m$^3$ (or 0–1 particle sized 1, 3, 5, or 10 μm as detected by the particle counter), the filters were inactivated, but the fans continued working. Fig. 3 features data obtained starting from this point of the experiment. After the filtration system was switched off, aerosol was generated from a 10% saccharose solution in distilled water for 5 min by the Comp Air NE-C28 compressor nebulizer (Omron Healthcare Co., Ltd.; Japan) placed inside the chamber. Particles sized 1, 3 and 5 μm were counted inside the chamber using the counter of submicron particles 4705. The counter reported an average number of particles per 1 min for 16 minutes in a row. In the first operating mode (precipitation of unstirred aerosol) the fans were turned off, the filtration system inactivated, and aerosol particles counted for 11 minutes. In the second operating mode (precipitation of stirred aerosol) the fans were turned on, filtration switched off, and particles counted for 11 min. Additionally, at this stage of the experiment we introduced the aerosol sampler designed at MEPhI and counted aerosol particles while the device was working. In the third mode (air decontamination from stirred aerosol) the fans were turned off, filtration inactivated, and particles counted for 11 minutes.

3. Comparing the efficiency of aerosol collection of the two aerosol samplers (Test 3)

To evaluate the efficiency of polydisperse aerosol collection, we prepared 100 ml of a 10% saccharose solution in distilled water containing fluorescein sodium salt at a final concentration
of 1 µM. This amount was sufficient to run all the tests we planned. Fluorescence intensity of the collected tracer was measured on the Qubit 3.0 Fluorometer (Invitrogen; USA) in relative fluorescence units at 470 nm excitation and 510–580 nm emission wavelengths in 0.6 ml test tubes (SSI; USA) containing carbonate buffer (pH 9.6) (C3041; Sigma; Germany) measured fluorescence intensity. If the measured value exceeded the background fluorescence of empty test tubes, the cyclone collector was washed again. Once the anticipated fluorescence intensity was reached, the ventilation system was turned off.

Aerosol was generated inside the chamber by the compressor nebulizer Comp Air NE-C28 for 5 minutes. Prior to each test, the device was washed and wiped dry with a lint-free cloth. Then 4 ml of the saccharose solution containing sodium fluorescein were loaded into the nebulizer. The aerosol sampler was turned on simultaneously with the nebulizer and collected aerosol particles for 6 min, i.e. the sampling process continued for another minute after aerosol generation was terminated. After samples were taken to the lab for the analysis, the cyclone collector was washed automatically with water and then the impactor turned on. After that, control air samples were collected for 6 minutes. For the analysis, we took 270 µl of the sample, mixed them with 30 µl 10x carbonate buffer (pH 9.6) (C3041; Sigma; Germany) and measured fluorescence intensity. If the measured value exceeded the background fluorescence of empty test tubes, the cyclone collector was washed again. Once the anticipated fluorescence intensity was reached, the ventilation system was turned off.

Table 1. Integrity tests of HEPA filters installed in the chamber

| HEPA filter                        | Number of 0.3 µm particles (N_c) before air filtration | Concentration of aerosol particles per cm² (C) before air filtration C_c = N_c / q_v * T_p | Number of particles sized 0.3 µm or larger after air filtration | The highest detected number of particles sized 0.3 µm or larger exiting the filter scan (N_cscan) ≤ C_c * P_v, * q_v, * T_s | Threshold |
|-----------------------------------|--------------------------------------------------------|------------------------------------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|-----------|
| Inlet filter                      | 50109                                                  | 1061                                                                                     | 130                                                             | ≤ 18779                                                        | Not exceeded |
| First stage exhaust filter        | 91343                                                  | 1935                                                                                     | 4493                                                            | ≤ 13701                                                        | Not exceeded |
| Second stage exhaust filter       | 49624                                                  | 1051                                                                                     | 281                                                             | ≤ 18602                                                        | Not exceeded |

Table 2. Rate of dust particle collection by the aerosol sampler (National Research Nuclear University MEPhI)

| Time elapsed from the start of the test (min) | 1 µm particles | 3 µm particles | 5 µm particles |
|-----------------------------------------------|----------------|----------------|----------------|
| 5                                             | 56829268       | 100%           | 1289540        | 100%           |
| 7                                             | 41036585       | 72%            | 555680         | 43%            |
| 8                                             | 21097561       | 37%            | 160966         | 12%            |
| 10                                            | 11707317       | 21%            | 51038          | 4%             |
| 11                                            | 6951220        | 12%            | 17032.8        | 1%             |
| 12                                            | 4512195        | 8%             | 6402.4         | 0.5%           |
Dynamics of air decontamination from 1 to 10 µm particles (Fig. 3) in the Laminar-C-1.5 Class III Biosafety Enclosure after 5 minutes of aerosol generation in different operating modes.

Table 3 demonstrates the efficiency of polydisperse aerosol collection evaluated from the fluorescence intensity of the tracer. Fluorescence expressed in relative fluorescence units was normalized to the volume of the released liquid sample for each device. The prototype of the aerosol sampler developed at the National Research Nuclear University MEPhI has an average rate of collection similar to that of SASS; based on the results of 5 measurements, the difference is insignificant ($p > 0.05$; Student’s T-test applied). For each of the compared aerosol samplers 4 washes in the ventilated environment were enough to remove the fluorescent tracer and reach the level of background fluorescence.

V. Discussion of the results

There are a few factors complicating the study of properties exhibited by biopathogens in ambient air. First, bioaerosols must be generated in enclosed laboratory spaces to prevent damage to the environment and people. Seconds, such enclosures must have an built-in system for rapid and efficient air decontamination and disinfection after the experiment. Third, they must satisfy the strict sanitation and safety requirements set by state agencies. Finally, our high-tech age demands that such chambers should be made from cutting-edge environment-friendly materials and equipped with sensors to...
monitor pressure, temperature, air flow rate, air cleanliness, and other parameters inside the chamber and have electronic panels for control and data input [8–10]. All of these factors have to be accounted for in the design of the end product. Given that it is not intended for mass production, the final price can be really formidable.

In Russia, such workstations are available in the shared facilities of big research institutions. A good example is an enclosure for static and dynamic aerosol measurements designed at the State Research Institute for Biological Instrumentation to test the technical parameters of biosafety systems in the controlled environment at temperatures ranging from -20 to +40 °C and relative humidity of 30 to 95% [11]. This huge enclosure (static chamber sizes are 8 and 26 m²) is a perfect space to experiment with pathogenic aerosols and airborne microorganisms. Sadly, for technical reasons the Institute cannot work with pathogens included in the hazard groups 3 and 4 which seriously limits the range of tested bioaerosols to their simulators. Although a series of minor experiments can be conveniently conducted here, the situation does not favor long-term extensive research, which is very unfortunate given construction costs.

Class III biosafety cabinets are cheaper and, therefore, more available. They protect the operator and the environment from contamination by hazardous aerosols resulting from manipulations with pathogenic agents and also protect the agents inside the chamber from external contamination. Cabinets are intended for one or two operators and their internal working space is quite small (> 1 m³).

The experimental workstation described and successfully tested in this study is a trade-off between the two. This Laminar-C-1.5 Class III Biosafety Enclosure (380.150.01) was designed to test the devices for testing and analyzing model pathogenic bioaerosols. Class H14 HEPA filters installed in the chamber capture over 99.995 % of generated particles sized >0.3 µm [12]. The primary application of these filters is air decontamination from radioactive particulate matter with 99.97 % efficiency. They have a few drawbacks. The majority of (but not all) bacteria are from 0.4 (Proteus mirabilis) to 10 µm (Clostridium perfringens) in size; pathogenic fungi vary in size from 1 (Pneumocystis jirovecii) to 20 µm (Fusarium sp.). Viruses fall into the range from 0.02 (Rhinovirus) to 0.3 (Mumps virus) µm [13]. In addition, for some pathogens the infective dose is really tiny, such as <10 bacterial cells for C. burnetti that causes Q fever. These 10 cells may well be part of those 0.005 % of the particles that are not retained by the filter in the experiment where the number of the sprayed bacteria is 10² CFU or higher [14]. Studies of HEPA filters involving the MS2 phage have shown that filtration efficiency is directly dependent on the air flow rate [15]. Thus, the choice of filters is largely determined by the task set for the researcher. Our Laminar-C-1.5 Class III Biosafety Enclosure is equipped with two consecutive exhaust HEPA filters (the calculated particle penetration value is 3 x 10⁻⁵%), and is capable of maintaining the optimal air flow rate, ensuring sufficient protection in the experiments involving high concentrations of biopathogens (up to 10⁶–10⁷ CFU/m³). The conducted tests (Table 1) prove that the filters satisfy the state requirements for integrity and efficiency and that the chamber can be used for the majority of standard tasks. Tests of the rate and efficiency of air decontamination from 1, 3 and 5 µm particles have shown that aerosol concentration remains more or less stable for quite a long time (11 minutes) if the systems of filtration and ventilation are turned off. When the fans inducing air circulation in the chamber are turned off, the concentration of particles of all three sizes slightly decreases within the same time interval. The system of air decontamination has demonstrated high rate and efficiency by removing all particulate admixtures from the air in 3 to 6 minutes depending on the size and number of the particles generated in the chamber. In test 3 some parameters of the aerosol sampler developed at the National Research Nuclear University MEPhI were compared to those of its foreign counterpart.

CONCLUSION

Laminar-C-1.5 Class III Biosafety Enclosure (380.150.01) is a high-quality workstation for aerosol tests that can be installed inside a lab. The volume of the work chamber is 4.275 m³, which is sufficient for the first- and second-level tests involving the majority of biopathogens. The workstation can be used to test aerosol samplers and study bioaerosol behavior at various concentrations in static and dynamic modes.

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Table 3. Efficiency of polydisperse aerosol collection

| Aerosol sampler | Average sample volume, ml | Average RFU/ml considering error of measurement | Relative to SASS, % |
|-----------------|--------------------------|-------------------------------------------------|-------------------|
| MEPhl           | 7.5                      | 1441 ± 98                                       | 97.7              |
| SASS            | 4.5                      | 1475 ± 122                                      | 100               |

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