HIGH-PERFORMANCE AEROSOL SAMPLER WITH LIQUID PHASE RECIRCULATION AND PRE-CONCENTRATION OF PARTICLES

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Testing the surrounding environment for the presence of biogenic aerosols is crucial in ensuring its safety for the population. It is often necessary to collect aerosol samples from large areas in short time, which demands excellent particle collection efficiency, a sufficient incoming air flow rate and a capacity to maintain the viability of the collected samples. Below we present the aerosol sampler with a high volumetric flow rate based on a two-stage particle concentration algorithm and consisting of a virtual impactor and a cyclone concentrator with a recirculating liquid phase. We provide all necessary calculations and an algorithm for modeling impactor parameters. The sampler was tested using dry and liquid formulations dispersed into the particles of 0.5 to 5 μm in diameter. We demonstrate that at volumetric flow rates over 4,000 l/min efficiency of particle collection into the liquid phase at a volume of 10 ml makes over 20% of the total aerosol mass and at volumetric flow rates over 300 l/min this value is over 60%. The proposed device maintains viability of the collected microorganisms. The sampler is portable, with flexible settings for sampling and cleaning, and can be controlled remotely over the network.

Keywords: aerosols, pathogens, efficiency, sampler, impactor, volumetric flow rate, cyclone

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ВЫСОКОПРОИЗВОДИТЕЛЬНЫЙ АЭРОЗОЛЬНЫЙ ПРОБООТБОРНИК С РЕЦИРКУЛЯЦИЕЙ ЖИДКОЙ ФАЗЫ И ПРЕДВАРИТЕЛЬНЫМ КОНЦЕНТРИРОВАНИЕМ

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Обнаружение биогенных аэрозолей является важной задачей при обеспечении безопасности жизнедеятельности человека в современных условиях. На практике часто требуется собирать аэрозоли с больших площадей за малый промежуток времени, что накладывает жесткие ограничения на эффективность пробоотбора, величину прокачиваемого в единицу времени объема воздуха и жизнеспособность собранного биоматериала. В работе представлены результаты по разработке и испытанию устройства отбора аэрозольных проб с высокой объемной скоростью и двухступенчатым концентрированием аэрозольных частиц — виртуального импактора и циклонного коллектора с рециркулирующей жидкой фазой. Приведены алгоритм и результаты расчета параметров импактора, результаты испытаний устройства на модельных сухих и жидких тест-препаратах для частиц размерами 0,5–5 мкм. Подтверждено, что при объемных скоростях пробоотбора выше 4000 л/мин эффективность отбора в жидкую фазу объемом до 10 мл составляет более 20% массовой доли распыленного аэрозоля, а при объемных скоростях выше 300 л/мин — более 60% массовой доли. Показано, что устройство сохраняет жизнеспособность отобранного биоматериала. Прооотборник реализован в портативном варианте, обладает возможностью настройки всех параметров отбора и очистки, а также управления по сети.

Ключевые слова: аэрозоли, биопатогены, эффективность, пробоотборник, импактор, объемная скорость, циклон

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Testing air for the presence of pathogenic, allergenic and immunogenic microorganisms is crucial in ensuring its safety for the population. Advances in biotechnology have added to the sources of contaminating aerosols, which now include genetically engineered microbial strains used in the production of pharmaceuticals, enzymes and synthetic foods [1]. Microbial concentrations in contaminated air can reach as high as 10^6 CFU/m^3 causing respiratory infections and

allergies in humans [2]. Another serious threat is posed by bioterrorism, which involves the intentional contamination of air with pathogens.

Traditionally, air sampling for bioaerosol detection and quantification is done using filters, impingers and impactors in which microorganisms go through a lot of stress caused by the sampling process itself and are unlikely to survive [3]. Microbial viability is critical when it comes to the sampling of microbiological flora. It is important to avoid applying unnecessary physical force on the collected microorganisms and to create conditions for maintaining their physiological properties. Here, great promise is held by liquid-based samplers [4] that separate microorganisms from their aerosol carriers and ensure accurate detection of individual microbial cells.

An air sampler for pathogen detection is expected to meet a number of elaborate requirements [5–8]. First, the volumetric air flow rate must be high enough to allow detection of low pathogen concentrations in reasonable time. Second, the capture of aerosol particles and the process of their concentration in a small liquid volume for further analysis must be efficient. Third, precipitation conditions must be gentle to allow survival of collected microorganisms and the sorption liquid must contain protective components. Finally, the aerodynamic drag has to be low, and the sampler is expected to produce little noise and have low energy consumption.

Devices for collecting aerosols from the surrounding air exploit different physical principles and have been around for quite a long time. They all have their drawbacks. Creating a sampler operating at a flow rate of over 3,000 l/min, with low levels of noise and energy consumption, capable of efficient pathogen capture and ensuring viability of captured microorganisms concentrated in small liquid volumes remains a challenge still awaiting a solution.

The aim of this work was to design a high-performance device for collecting and concentrating bioaerosols from the surrounding air and to test the obtained samples for pathogenic bacteria and viruses.

I. Design of the experiment

Our device exploits the principle of two-stage particle concentration and allows working with high volumetric flow rates. During the first stage, the captured particles are concentrated in the virtual impactor as the air flow coming through the inlet nozzle is forced to abruptly change its direction [9, 10]. The exiting air flow containing the concentrated particles follows the original direction of the incoming flow, but its rate is several times lower. During the second stage, the concentrated particles deposit on a recirculating liquid film of the cyclone [3, 11]. As the particles keep coming in, longer circulation time causes their concentration in the liquid to increase. The stages of the experiment are described below.

1. Creating a virtual impactor

A few preliminary calculations were done to compute the width and length of the impactor's inlet nozzle through which the air is sucked in and outlet nozzle through which the air containing concentrated particles is released at a decreased flow rate. In our calculations, the incoming flow rate ranged from 3,000 to 5,000 l/min, the size of the particles varied from 0.5 to 5 μm. According to [12], the Reynolds number and the ratio of the distance separating the inlet and the outlet nozzles to the width of the inlet nozzle determine the shape of the curve representing dependency of particle collection efficiency (expressed as percentage) on the aerodynamic diameter of the particles. The diameter corresponding to the collection efficiency of 50% is calculated according to the equation

$$d_{50} = \left(\sqrt{\frac{9\eta W}{\rho_p C_C U}} \right) \times \sqrt{Sk_{50}} \quad , (1)$$

where ρ_p is particle density; C_C is the Cunnigham slip correction factor accounting for the increase in the mobility of particles whose size is comparable to the gas mean free path; U is particle velocity; η is air/gas viscosity; W is nozzle width; S_{k50} is Stokes number corresponding to the diameter d_{50}. We aimed to select such nozzle widths that would ensure the Reynolds number

$$Re = \frac{\rho_{air} WU}{\eta}$$

in the range between 500 and 3,000 at a volumetric air flow below 5,000 l/min [12]. Based on the dependency of S_{k50} on Re calculated in [13], we determined the value of S_{k50} and then calculated the value of d_{50} according to the equation (1). This value cannot exceed the minimum required diameter of aerosols of 0.5 μm. In total, twenty different nozzle sizes were obtained with different values of the Reynolds number and d_{50}.

Next, we built a model of a virtual impactor body based on the calculated nozzle parameters and modelled the trajectory of aerosol particles in it. We also estimated distribution of particle velocities at each point of space in the impactor body covered by our calculations. For that, we used Solid Works 2014 (the system for automated modelling) and the Flow Simulation application.

During the third stage, we calculated the efficiency of particle collection at given parameters considering the obtained distribution of particle velocities. Calculations were done in the original software and the MathLab environment. The software estimated how a group of 100 aerosol particles relocated spatially as they travelled between the nozzles. Coordinates of every particle were calculated with due account of the centripetal acceleration. The centripetal acceleration is determined by the force (Stokes' law) resulting from the interaction between the aerosol particle and the air flow as it bends while traveling between the nozzles (Fig. 1). We assessed how well the particles "found" the outlet nozzle.

Finally, the joint performance of the virtual impactor and the cyclone concentrator was tested.

2. Creating a cyclone concentrator

To design a liquid-phase cyclone concentrator, we used calculations from [3]; they aid in measuring the efficiency of particle capture by the sorption liquid based on the height and radius of a cylinder in which the liquid circulates (Fig. 2). We hypothesized that for the cyclone the incoming flow rate would have the same value as the flow rate exiting from the virtual impactor. For liquid recirculation, a separate channel was introduced into the cyclone concentrator.

II. Testing the sampler

The fabricated virtual impactor and the cyclone were connected by a flexible air pipe and tested together and separately for the efficiency of aerosol collection. The tests were conducted at the facilities of the 48th Central Research Institute of the Ministry of Defense of the Russian Federation (Sergiev Posad), Gamaleya Research Institute of Epidemiology and Microbiology (Moscow) and in the Moscow Metro.

The tests conducted at the facilities of the 48th Central Research Institute for 10 days involved the use of a dry pathogen-free test formulation. The impactor was placed inside a static aerosol chamber; the cyclone was connected to the impactor by a flexible air pipe and placed outside the chamber. The concentration and size distribution of aerosol particles, as well as the total mass of the particles trapped in the sorption liquid, were measured by fluorescence and chemiluminescence. The efficiency of sampling was assessed relative to the KPK-3 sampler. Aerosol particles were generated in the static chamber from the dry pathogen-free test formulation by the pneumatic pulse generator. KPK-3 and May's 4-stage impactor were used to measure the integral mass concentration of aerosol and the distribution of particle sizes.

Another series of tests was conducted in the security check areas of Cherkizovskaya and Novokosino metro stations to compare the performance of our model with that of the SASS4000/2300 aerosol concentration device with a cyclone air sampler (Research International Inc.; USA). The collected samples were sent to the laboratory for the microbiological and biomolecular analyses to determine the composition of the captured microbial communities and to quantify them.

III. Microbiological analysis of the obtained samples

The following ready-to-use solid agar media were used: Columbia agar with defibrinated blood, Baird-Parker agar, Sabouraud dextrose agar w/ chloramphenicol, Endo agar, enterococci agar and lysogeny broth prepared on site (composition (g/l): 10 h g tryptone, 5 g yeast extract, 5 g NaCl, 17 g agar). Cultures were plated onto Petri dishes (CFGS; Russia). Each culture was plated onto 6 dishes containing different growth media. Before plating, the media were preheated to room temperature and dried in an incubator to remove condensed moisture.

Liquid samples were plated by pipetting (0.1 ml of the culture per Petri dish). The pipetted cultures were evenly spread across the medium surface with a sterile L-shaped spatula.

The cultures were incubated at 37 °C for 48 h. The cultures grown in Sabouraud agar were incubated at room temperature for up to 7 days when no visible culture growth was observed.

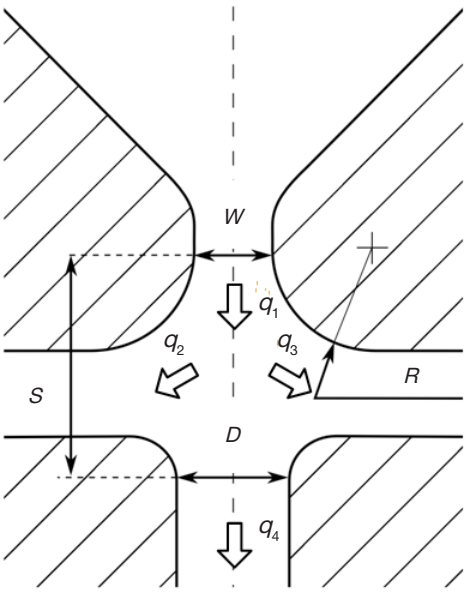

Fig. 1. The schematic of the virtual impactor (cross-section). *W* is the width of the inlet nozzle; *D* is the width of the outlet nozzle; *S* is the distance between the two nozzles; q_1 is the incoming air flow; q_4 is the exiting air flow containing concentrated aerosol particles; q_2 and q_3 represent the discarded flow

Grown colonies were counted in every dish and their morphological types (MTs) were described. Every MT received an identifier, and the colonies were photographed. Isolated colonies were reseeded onto fresh growth media for further identification and antibiotic susceptibility testing. Colonies with pronounced morphological features were preliminary identified to the genus level.

Sensitivity of the isolated cultures to antibiotics was determined by disc diffusion tests (Himedia; India). The panel of antibiotics included ampicillin, amoxiclav, cefoxitin, azithromycin, levofloxacin, gentamycin, amikacin, tetracycline, vancomycin, novobiocin, bacitracin, optochin and Mueller Hinton agar standardized for these purposes (CFGS; Russia).

Halos (zones of inhibition) around antibiotic discs were measured; their diameters were compared to the reference interval and assigned to one of three categories: r (resistant), s (susceptible) and i (moderately susceptible).

Tests were carried out at the facilities of Gamaleya Research Institute of Epidemiology and Microbiology in order to compare the performance of our system with that of the SASS4000/2300 aerosol concentration device with a cyclone air sampler (Research International Inc.; USA) using a test aerosol. Measurements were taken in the biosafety cabinet Laminar-S (Laminar systems; Russia). The liquid test formulation for aerosol generation was a 10% solution of saccharose in the carbonate buffer (pH 9.6) (C3041; Sigma; Germany) with fluorescein sodium taken at a final concentration of 1 μM. We compared the luminescence intensity of the sample collected for 5 min in the static chamber with the continuously generated test aerosol.

IV. Simulation of virtual impactor parameters and particle collection efficiency

We investigated the dependency of collection efficiency for the particles sized 0.5–5 μm in diameter on the parameters of the virtual impactor (Fig. 3–5). Collection efficiency was calculated as the ratio of the number of concentrated particles of a given size (the particles that made it to the outlet nozzle) (Fig. 1) to the number of particles present in the incoming air flow.

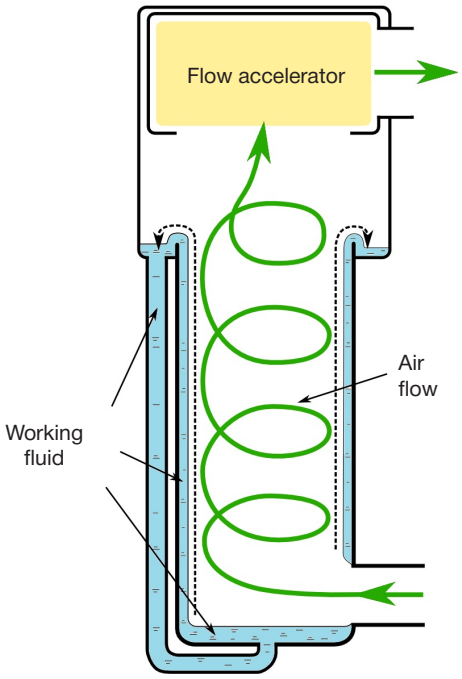

Fig. 2. The schematic of a liquid-phase cyclone concentrator

Collection efficiency rises from 18% to 37% for 0.5 μm-sized particles when the size of the inlet nozzle *W* goes down from 0.07 to 0.05 cm (Fig. 3). However, at *W* = 0.04 collection efficiency drops dramatically. This phenomenon was previously described in the literature [14] and means that the ratio of the inlet to the outlet nozzles should not be ignored: to achieve maximum effective collection, the inlet nozzle must be 30–40% smaller than the outlet nozzle.

At distances *S* between the inlet and outlet nozzles of 0.13 and 0.15 cm, collection efficiency reaches 27% for the particles

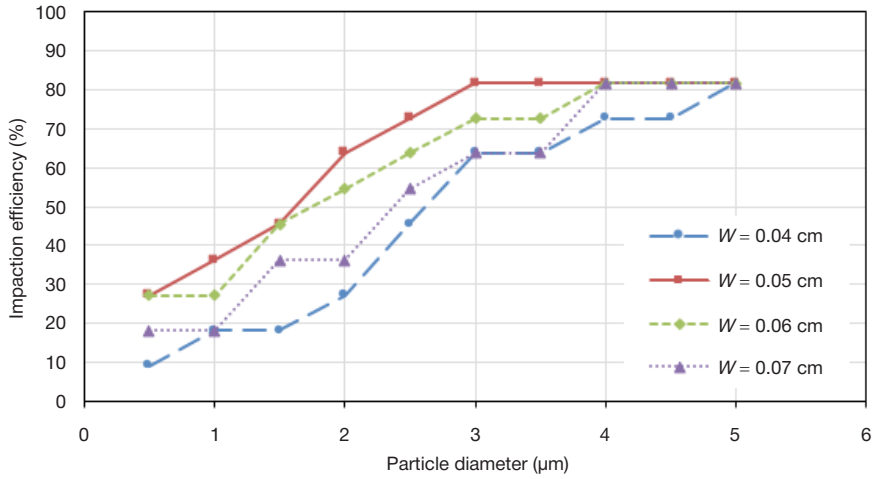

Fig. 3. Dependency of particle collection efficiency on aerosol particle size at various inlet nozzle widths *W* (Fig. 1) of the virtual impactor. The outlet width is 0.07 cm

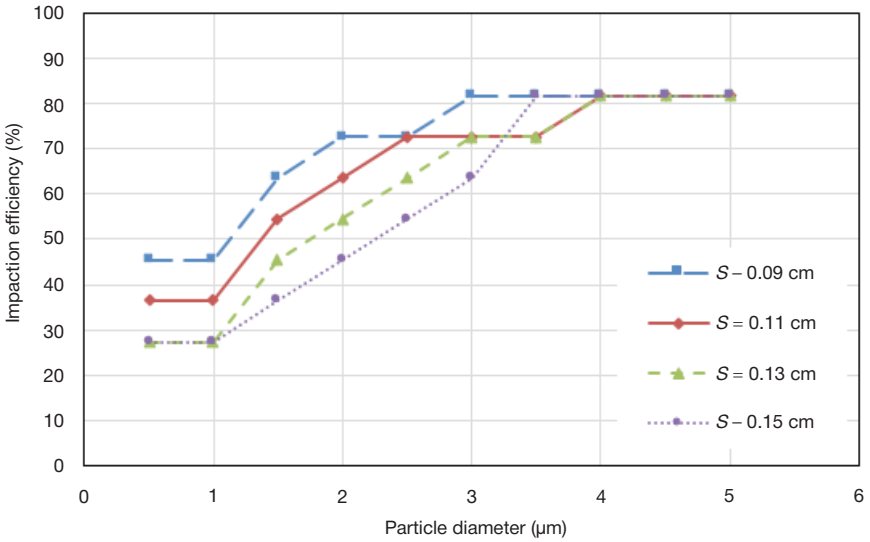

Fig. 4. Dependency of liquid collection efficiency on the aerosol particle size at various distances *S* between the inlet and outlet nozzles (Fig. 1) of the virtual impactor. The width of the inlet and outlet is 0.07 cm

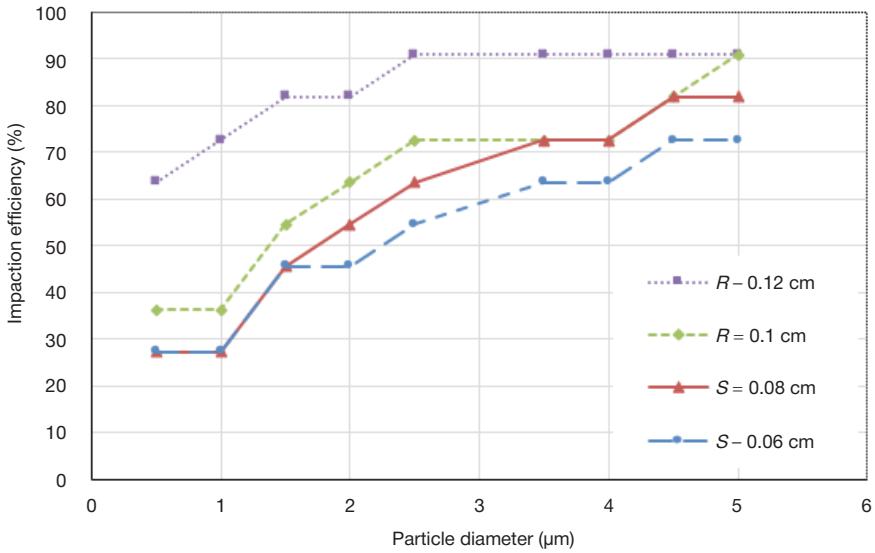

Fig. 5. Dependency of liquid collection efficiency on the corner radius *R* of the inlet nozzle. The width of the inlet and outlet nozzles is 0.07

sized from 0.5 to 1 μm (Fig. 4). When this distance shrinks to 0.09 cm, collection efficiency increases to 45%. Importantly, the anticipated efficiency for the particles over 4 μm in size at all possible *S* values does not exceed 82%.

As the corner radius *R* of the nozzle dips to 0.06 cm, particle collection efficiency drops for 1.5–5 μm-sized particles (Fig. 5). When the radius increases to 0.12 cm, 0.5 μm-sized particles are collected more efficiently (64%); for the particles of 2.5 μm in diameter and larger, collection efficiency is as high as 91%. However, one should be careful with the corner radius because of the risk of turbulence between the nozzles at high radius values.

Our calculations yielded a few parameters determining impaction efficiency (transfer of aerosol particles into the liquid phase), including the tube radius R = 42 mm, its height H = 100 mm, and the diameter of the inlet nozzle =15 mm at the volumetric flow rate Q = 350 l/min.

V. Simulation results and device tests

Fig. 6 demonstrates the schematic of the air sampler. The sampling device consists of a virtual impactor connected to a cyclone by an air pipe.

The cyclone is the control module of the device. Control is implemented via a sensor screen and is fully automated. Sampling occurs in a series of steps constituting a full cycle. The cycle includes supply of liquid from a tank into the cyclone, sampling by pumping air through the device, release of a liquid phase for the analysis, and washing of the cyclone. The duration of cycles and the values of volumetric flow rates can be regulated by an operator. The volume of the liquid phase returned by the device for further analysis ranges from 2.5 to 10 ml. The device can connect to a network via the RS-485 interface.

Table 1 demonstrates the results of the tests conducted at the facilities of the 48th Central Research Institute of the Ministry of Defense of the Russian Federation. Collection efficiency was measured for the dry test formulation and reached as high as 20% of the total mass of the generated aerosol particles. The volumetric flow rate of the device was estimated to be 100 times higher than that of the KPK-3 sampler ensuring 100% particle collection. The cyclone disconnected from the impactor collected up to 61% of the total mass of the particles at a flow rate 6 times higher than that of KPK-3.

Sixty-four samples collected in the Moscow metro were forwarded to the laboratory for the microbiological analysis. Forty-eight morphological types of microorganisms were isolated from the samples. Those microorganisms represented microbial communities inhabiting the air and surfaces of the metro stations. Our air sampler did not differ significantly from the SASS system and the control nanofilters (high-density filters) in terms of the number of microbial morphological types isolated from the collected samples (Fig. 7).

Four of five studied bacterial strains isolated from the samples collected in the Moscow metro were antibiotic-resistant. The strain *St. haemolyticus* MT22 demonstrated multiple drug resistance to macrolides and fluoroquinolones. The strain *Streptococcus viridans* MT8 was multidrug-resistant to macrolides, aminoglycosides, and inhibitor-protected ß-lactams (Table 2). These findings suggest that our device can be used to monitor the spread of antibiotic resistance in hospitals and the surrounding environment in general.

Quantification of total DNA isolated from the samples using the commercial PureLink™ Microbiome DNA Purification Kit (Invitrogen; USA) also showed the absence of any obvious advantage of the tested sampling systems over each other. Our device and SASS surpassed the performance of the nanofilter by two orders of magnitude.

The experiments involving the liquid test formulation conducted at the facilities of Gamaleya Research Institute of Epidemiology and Microbiology demonstrated that our sampling device ensures particle collection of 96% relative to the SASS system (5 tests were conducted; CI was 0.95).

VI. Optimization of the air sampler

Parameter simulation and device testing show that the main challenge is posed by the concentration of aerosol particles < 1 μm in diameter. Collection efficiency of 50% (d50) for such particles requires narrow inlet nozzles. Narrowing the nozzle from 0.5 to 0.4 mm causes a 1.5-fold decline in the incoming flow rate and therefore negatively affects collection efficiency. To maintain the sufficient incoming air flow rate, pressure difference generated by the fan needs to be increased accordingly, which will increase energy consumption and the size of the device. It is reasonable to assume that the real achievable linear flow rate of the incoming air cannot be more than 100 cm/s for small particles < 1 μm in size and that the width of the nozzle cannot be less than 0.5 μm. In our device the volumetric flow rate does not exceed 4,500 l/min when the device operates at maximum power.

Efficiency of particle collection into the liquid phase by the cyclone can be increased by spraying finely dispersed water droplets in the inlet. Optimization is also required for the balance between the aerodynamic drag in the outlet of the virtual impactor and the inlet of the cyclone concentrator, which we did not attempt in the course of our experiment. Remote control of the device and its settings may also be a useful feature.

CONCLUSION

This work presents calculations for the fabrication of an aerosol sampler for the particles of 0.5 to 5 μm in diameter, operating at a high volumetric air flow and ensuring efficient particle collection in the liquid phase. The device that successfully passed a series of tests can reach the volumetric flow rate of 4,500 l/min, demonstrates the particle collection efficiency of 20% (of the total particulate mass) at the flow rate over 4,000 l/min and the particle collection efficiency of up to 61% at the volumetric flow rate over 300 l/min. The device can collect

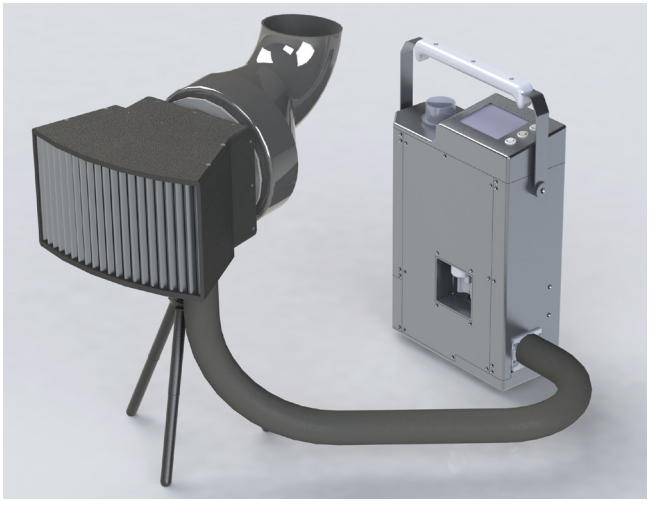

Fig. 6. The Cyclon-Bio device assembled. *On the left*: the impactor. *On the right*: the cyclone concentrator

Table 1. Test of the sampler performance using a test formulation (Central Research Institute of the Ministry of Defense of the Russian Federation)

Device	Integral concentration of the test formulation, mg/l (5 test, CI of 0.95)	Duration of sample collection, min	Volumetric flow rate, 1/min	Collection efficiency, %
Aerosol sampler	$(1.91 \pm 0.18) \cdot 10^{-3}$	2	4325	16 ± 1.5
	$(1.70 \pm 0.16) \cdot 10^{-3}$	2	4325	20 ± 2.1
Cyclone concentrator of the aerosol sampler	$(3.75 \pm 0.35) \cdot 10^{-3}$	2	325	61 ± 14
	$(2.91 \pm 0.27) \cdot 10^{-2}$	2	325	48 ± 125
KPK-3 sampler, collection efficiency control	$(3.75 \pm 0.36) \cdot 10^{-3}$	2	50	100.0
	$(2.91 \pm 0.28) \cdot 10^{-2}$	2	50	100.0

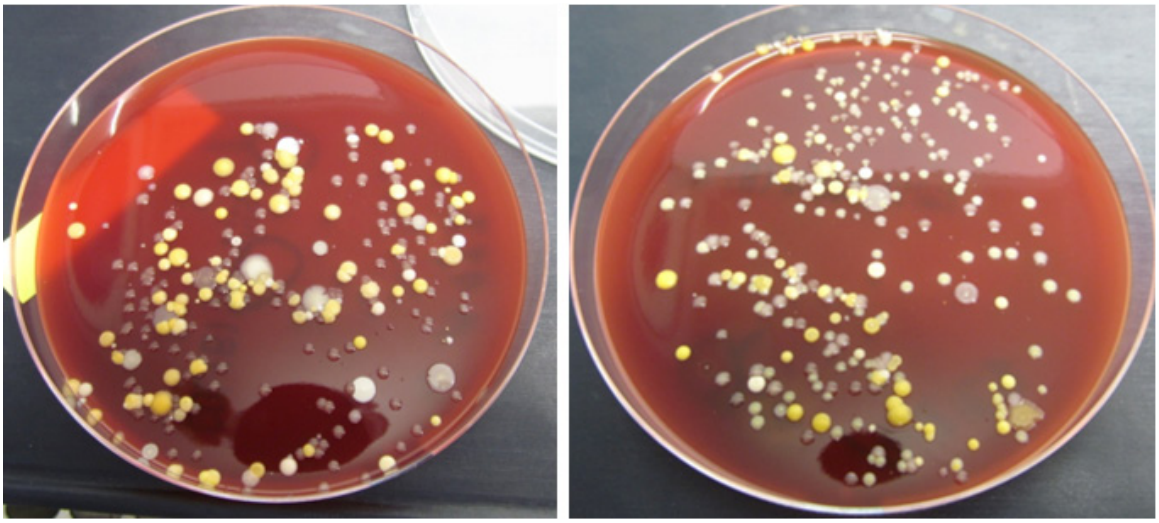

Fig. 7. Overnight culture of the samples collected at Novokosino metro station during the morning rush hour and plated onto blood agar. *On the left*: sample A123 collected by the SASS sampler. *On the right*: sample A223, Cyclon sampler (MEPhI)

Table 2. Testing antibiotic susceptibility of the collected strains

Antibiotic	MT 11 (*Staphylococcus haemolyticus*)	MT 12 (*Staphylococcus saprophyticus*)	MT 22 (*Staphylococcus haemolyticus*)	MT 24 (*Staphylococcus aureus*)	MT 8 (*Streptococcus viridans?*)
Ampicillin	**R**	S	S	S	I
Amoxiclav	n/a	n/a	n/a	n/a	**R**
Cefoxitin*	S	S	S	S	n/a
Azithromycin	S	**R**	**R**	S	R
Levofloxacin	S	S	**R**	S	S
Gentamycin	S	S	S	S	**R**
Amikacin	S	S	S	S	**R**
Tetracycline	S	S	S	S	S
Vancomycin	S	S	S	S	S
Novobiocin	S	R	S	S	n/a
Bacitracin	n/a	n/a	n/a	n/a	R
Optochin	n/a	n/a	n/a	n/a	R

particles in the range between 0.5 and 5 μm. The virtual impactor weighs 7.2 kg, and the cyclone concentrator weighs 5.6 kg. The device operates at 220 Volts AC and 24 and 12 Volts DC. The device is dust- and water-proof. Its performance is no inferior to that of the world's best air samplers. Over 90% of its components are made in Russia. The device can be used in public transport, at customs, border checkpoints or other public places to test the air for possible contamination and carry out environmental monitoring. It can also be installed in healthcare facilities and research institutions of the Ministry of Healthcare and the Ministry of Defense of the Russian Federation.

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