Lodz University of Technology
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Łódź, 18.09.2020

Research report

„Assessment of the effectiveness of air disinfection using a flow device prototype labelled "Respiray Device" for OÜ Respiray, Mõisa 4, 13522 Tallinn, Estonia

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Research aim:

The aim of tests was assessment of the effectiveness of a flow device prototype labelled "Respiray Device" for air disinfection provided by OÜ Respiray, Mõisa 4, 13522 Tallinn (Estonia) in model conditions using a bioaerosol generation system. Moreover, the concentration and dimensional distribution of the bioaerosol particles was assessment.

Methodology:

Microorganisms

Three bacteria species characterized by different physiology of growth were tested. Description of tested bacteria is presented in Table 1.

Table 1. Characteristics of tested bacteria strains

Microorganisms	Morphology	Collection Reference Number	Inoculum Density, CFU/mL
Staphylococcus aureus	Gram-positive cocci	ATCC 6538	$7.80\times10^{8} \pm 1.06\times10^{8}$
Escherichia coli	Gram-negative rods	ATCC 11229	$1.63\times10^{9} \pm 1.53\times10^{8}$
Escherichia coli	Gram-negative rods	ATCC 10536	$4.07\times10^{9} \pm 2.52\times10^{8}$

ATCC: American Type Culture Collection

Inoculum suspension preparation

Inoculum suspension for each tested strain was prepared. In order to obtain bacterial inoculum, 600 mL of sterile TSB medium (Tryptic Soy Broth (Merck, Germany) were inoculated with *S. aureus* ATCC 6538 or *E. coli* ATCC 11229 or *E. coli* ATCC 10536. Next the culture was incubated at 37°C±2°C for 24 h. The number of bacteria in the inoculum suspensions were determined by culture method. Serial dilutions were performed in saline solution and flooded with semi-solid TSA microbiological medium (Tryptic Soy Agar, Merck, Germany) After incubation at 37°C ±2°C for 24 h, colonies were counted, considering the dilution and

volume of the inoculum. The density of inoculum suspensions ranged 7.80×10^8 - 4.07×10^9 CFU/mL (Table 1).

Bioaerosol generation

Bacterial suspensions was used as the bioaerosol, which was passed through the tested device, and the microbiological filter was set up based on the schematic shown in Figure 1. Bacterial cultures were centrifuged at 5000 rpm for 10 min in order to separate the cells from the medium. The medium was removed, whilst bacteria were suspended in 600 mL of sterile saline solution (0.85% NaCl). Bacterial suspension was mixed on a magnetic stirrer; all steps were performed under aseptic conditions. The suspension was transferred into a sterile container-atomizer and connected to the equipment. The research was performed in a chamber with laminar air flow equipped with HEPA filters and a UV lamp. The tests consisted of dynamic generation of bioaerosol by an atomizer, which was mixed with a stream of dry air. This was subsequently directed into a system of filters installed in a tightly sealed setup. The tested flow device (Phot.1) was installed in the system between the in-line desiccator and the test chamber (Fig. 1).

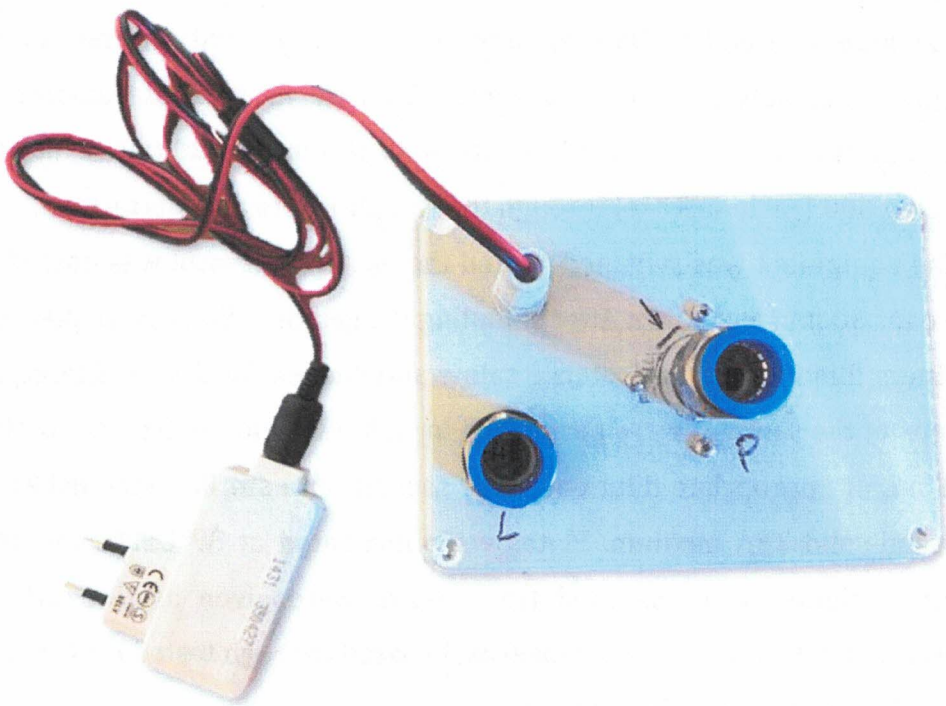

Photo 1."Respiray Device" (OÜ Respiray)

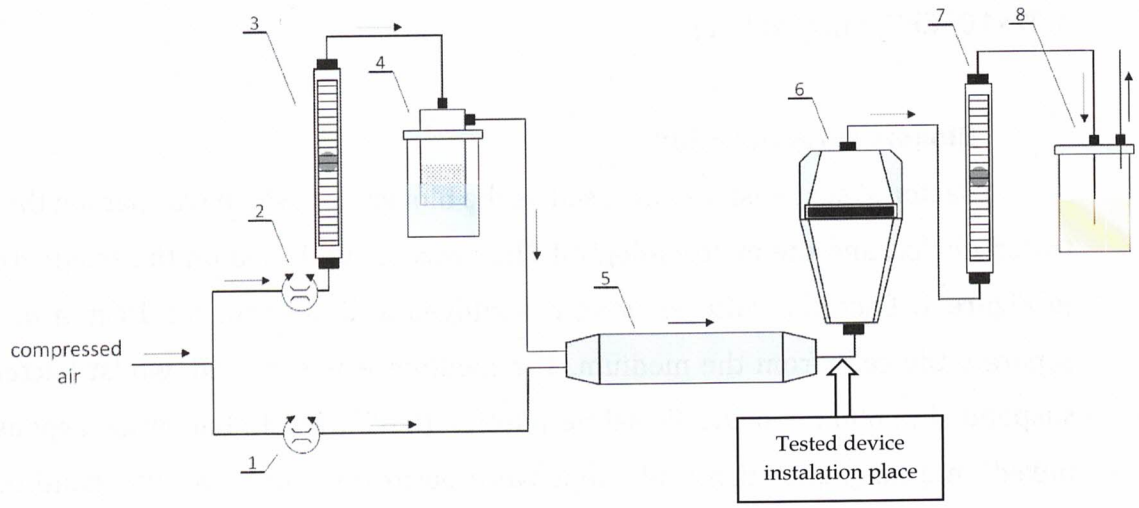

Fig 1. The scheme of the set-up used for bioaerosol generation 1, 2 – valves for precise air flow regulation compressor, 3,7 – air flow meter, 4 – Collison atomiser, 5 – in-line desiccator, 6 – test chamber, 8 – outlet of bioaerosol

The flow rate of the bioaerosol was 30 L/min and was controlled by a system of rotameters. The microbiological filter was used for quantitative analysis of the number of retained bacteria. An 80 mm gelatin microbiological filter (Sartorius, Germany) with a 3 μm pore size and 99.9999% retention rate was used for the study. A control experiments to determine the number of bacteria in 1 m^3 of bioaerosol generated under experimental conditions (flow rate of 30 L/min over 20 min, which corresponds to passing 600 L of bioaerosol through the sample) were also performed.

The equipment was switched on and the bacterial aerosol was passed through the sample for 30 min each time. After sampling the gelatine filters were placed into plastic containers filled with 50 mL sterile saline and shaken for 5 min. Subsequently, serial dilutions of the samples were performed in saline solution (from 10^3 to 10^8) and 1 mL or 0.1 mL of appropriate dilutions were seeded onto sterile Petri dishes and flooded with semi-solid TSA medium. Plates were incubated at 37°C±2°C for 24 h, and the resulting colonies were counted (the results were given as CFU/m^3). Number of bacteria was determined in the bioaerosol passed through tested device turned on and turned off in 3 independent replicates.

Bioaerosol particles measurement

The concentration and dimensional distribution of the bioaerosol particles was measured with the DustTrak ™ DRX Aerosol Monitor 8533 portable laser photometer (TSI, Shoreview, MN, USA), which allowed for simultaneous

measurements of the mass concentration of aerosol particle size fractions with diameters from the ranges (0.1 μm, 1 μm> , (1 μm, 2.5 μm>; (2.5 μm-4 μm>, (4 μm-10 μm>. Measurements were carried out continuously with a sampling interval of 1 s for 3 min. Analysis were performed for bioaerosol generated from all tested microorganisms suspensions.

Mathematical Analysis

Following the Grubbs test and after rejecting uncertain results, six results were selected for statistical analyses. The arithmetic means and standard deviations of the number of microorganisms grown on plates were calculated using Microsoft® Excel.

Differences between the numbers of bacteria before and after disinfection process were analysed using One-Way Analysis of Variance (ANOVA). Differences were considered significant at $p < 0.05$. All data were analyzed using the Origin 6.1 software (OriginLab Corporation, Northampton, MA, USA).

The percentage of bacteria reduction after disinfection (R) was calculated according to the equation:

$$R = \frac{A - B}{A} \times 100\%$$

where: R – percentage reduction of bacteria after disinfection; A – the number of bacteria on gelatine filter without device work (without disinfection); B – the number of bacteria on gelatine filter after device work (with disinfection);

Evaluation scale of the disinfection effectiveness was established:

R ≥ 99% - high antibacterial activity;

R = 90-98% - average antibacterial activity;

R = 50-89%- low antibacterial activity;

R < 50- lack of antibacterial activity.

Results:

In the figure 2 the total mass concentration of biological particles obtained in set-up for bioaerosol generation is presented. In all cases the concentration was stable during the measurement. There were differences in concentration which can be attributed to the specifics of the tested bacteria species.

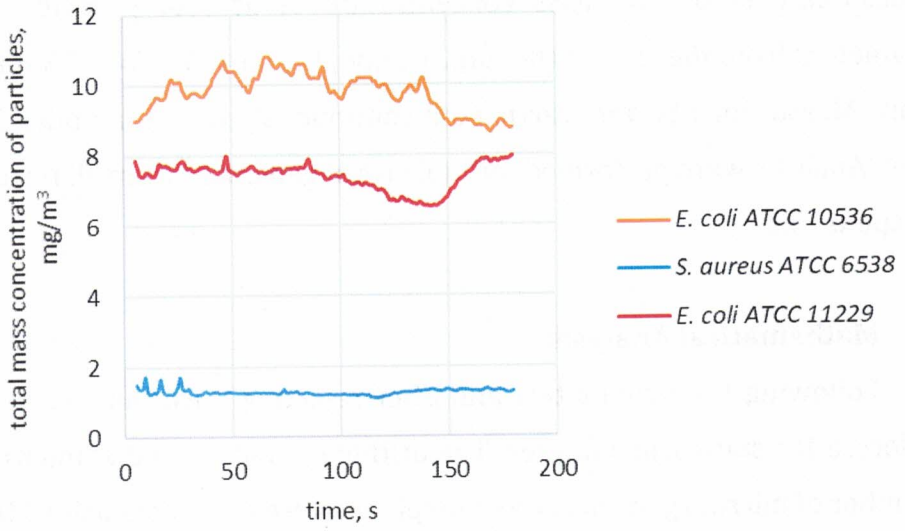

Figure 2. The total mass concentration of biological particles obtained in set-up for bioaerosol generation

In Table 2 and Figure 3 the size segregated mass concentrations are presented for tested species.

Table 2. Size segregated mass concentrations of bioaerosol obtained from tested bacteria

Mass concentration, mg/m³	Size fraction, µm		*E. coli* ATCC 10536	*E. coli* ATCC 11229	*S. aureus* ATCC 6538
	<1	M	9.66	7.34	1.26
		Min	8.56	6.45	1.11
		Max	10.70	8.95	1.53
	(1, 2.5>	M	0.11	0.09	0.00
		Min	0.10	0.08	0.00
		Max	0.10	0.12	0.02
	(2.5, 4>	M	0.01	0.00	0.01
		Min	0.00	0.00	0.00
		Max	0.00	0.04	0.05
	(4,10>	M	0.00	0.02	0.00
		Min	0.00	0.00	0.00
		Max	0.00	0.22	0.09

Min – minimum; Max – maximum; M - mean

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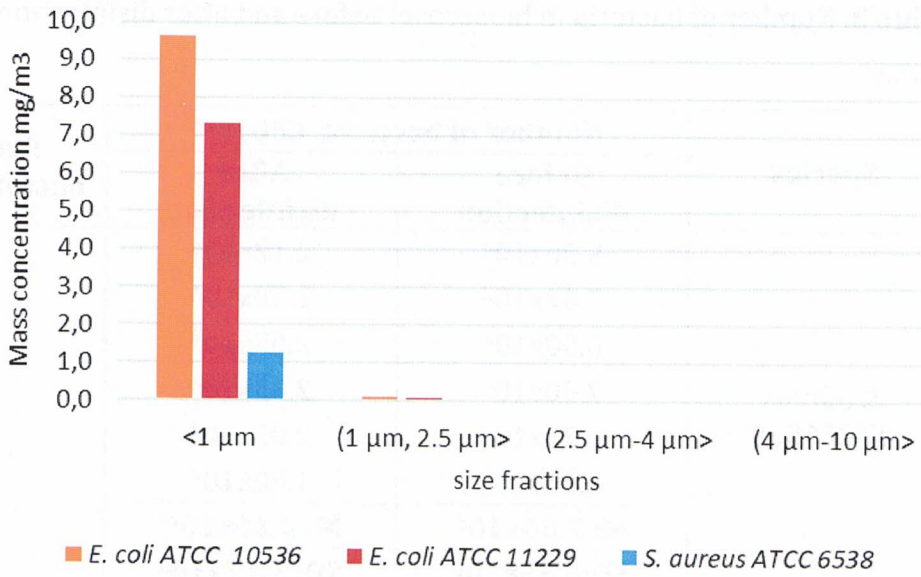

Figure 3. The size segregated mass concentrations of generated bioaerosol particles

As a result of the tests, it was found that in all tested cases the size of the tested particles does not exceed 1 µm, which corresponds to the literature data on the size of single cell of *E. coli* and *S. aureus*.

In Table 3 and Figure 4 the numbers of bacteria in bioaerosol before and after disinfection with tested device are presented

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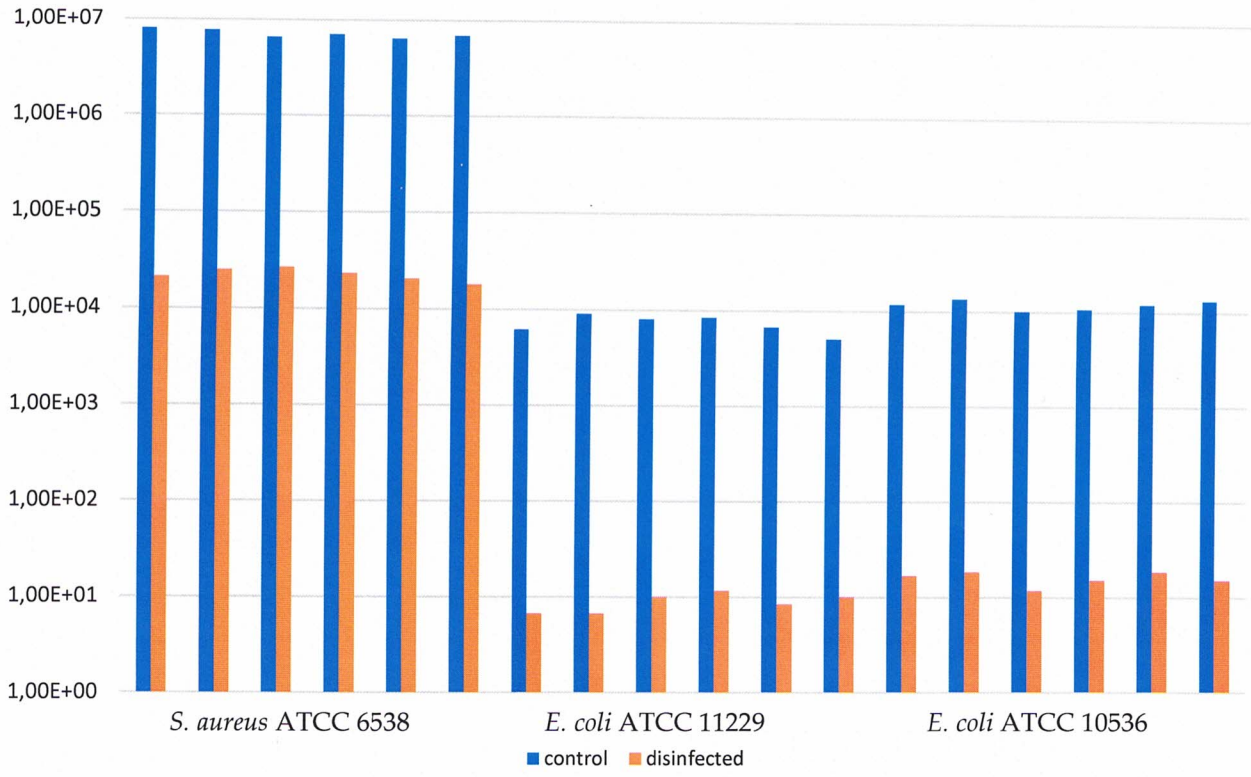
Table 3. Number of bacteria in bioaerosol before and after disinfection with tested device

Species	Number of bacteria, CFU/m^3		Reduction of bacteria numer, %
	Before disinfection	After disinfection	
S. aureus ATCC6538	$8.00x10^6$	$2.12x10^4$	99.68
	$7.67x10^6$	$2.50x10^4$	
	$6.50x10^6$	$2.67x10^4$	
	$7.00x10^6$	$2.33x10^4$	
	$6.33x10^6$	$2.05x10^4$	
	$6.83x10^6$	$1.80x10^4$	
	M: $7.06x10^6$	**M: $2.24x10^{4*}$**	
	SD:$6.55x10^5$	**SD: $3.17x10^3$**	
E. coli ATCC 11229	$6.17x10^3$	$6.67x10^0$	99.88
	$9.00x10^3$	$6.67x10^0$	
	$8.00x10^3$	$1.00x10^1$	
	$8.33x10^3$	$1.17x10^1$	
	$6.67x10^3$	$8.33x10^0$	
	$5.00x10^3$	$1.00x10^1$	
	M: $7.19x10^3$	**M: $8.89x10^{0*}$**	
	SD: $1.51x10^3$	**SD: $2.02x10^0$**	
E. coli ATCC 10536	$1.17x10^4$	$1.67x10^1$	99.86
	$1.33x10^4$	$1.83x10^1$	
	$1.00x10^4$	$1.17x10^1$	
	$1.05x10^4$	$1.50x10^1$	
	$1.18x10^4$	$1.83x10^1$	
	$1.30x10^4$	$1.50x10^1$	
	M: $1.17x10^4$	**M: $1.58x10^{1*}$**	
	SD: $1.32x10^3$	**SD: $1.53x10^0$**	

M: mean; SD: standard deviation. * - number statistically significant different (ANOVA: $p < 0.05$) compared to number after disinfection

The number of viable bacteria in generated bioaerosol before device work ranged from to 7.19×10^3 CFU/m^3 to 7.06×10^6 CFU/m^3 depending on the particular species. The bacteria number was statistically significant lower ($p < 0.05$) after disinfection with tested device for all tested bacteria. The reduction of bacteria number was at high level from 99.68% to 99.88% which corresponds to a decrease of 2-3 orders on a logarithmic scale (Table 3, Fig. 4).

Figure 4. Number of bacteria in bioaerosol before and after disinfection with tested device



Conclusions:

Tested flow device prototype labeled "Respiray Device" (OÜ Respiray), showed high efficiency (R=99.68%-99.88%) in reduction the number of bacteria *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 11229 and *Escherichia coli* ATCC 10536 in bioaerosol (the size of the tested particles < 1 μm) under model conditions.

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