TECHNICAL REPORT

PAEROSOL decontamination efficacy towards influenza viruses surfaced on different materials. Optimization of the process effectiveness.

MATERIALS AND METHODS

1. Decontaminating agent

Electro-activated solution (EAS) was produced with the STEL unit (NPO "Ekran", Moscow) from solution of NaCl in distilled water and distilled water at the final concentration of NaCl - 1% and current intensity of 10-11 A. The content of active chlorine in EAS - (0.10 ± 0.01) % and pH =7.0 ± 0.2. Freshly prepared EAS was used without sterilization.

Herein, PAEROSOL is atomized EAS

2. Viruses

Influenza viruses: A/Puerto Rico/8/34 (H1N1), the vaccine A/NIBRG-14 (H5N1) and the wild a/Duck/Kurgan/5/05 (H5N1) strains were used. The viruses were from the depository of the Research Institute of Influenza, RAMS (WHO Center for Influenza in RF).
The viruses were propagated in 10-12-day chicken embryonated eggs for 48 hours at 36°C, and the infective allantoic fluid was withdrawn and used for contamination. For the experiments on virus inactivation, the allantoic fluid containing 10^{7} - 10^{8} EID_{50} was used.

3. Titration of virus

Virus titers in control and experimental samples were determined using the growing 10-12-day chicken embryos.

3.1 Virus samples were subjected to 10-fold serial dilutions in physiological solution ranging from 10^{-1} to 10^{-9}.

3.2 Embryonated eggs were inoculated with 0.2 ml of the virus dilutions via a syringe. After inoculation the eggs were sealed with paraffin and incubated in a thermostat for 48 hrs at 36°C. 2 embryos were used per each virus dilution.

3.3 After termination of the incubation, the embryos were cooled for a night at 4° C, the shells were opened up, and the allantoic fluid was sampled with a serological pipette. The sampled fluid was dispensed over the wells of a round-bottomed titration plate (0.1 ml/well), and the equal volume of 1% suspension of chicken red blood cells in physiological solution

was added to each well. The plates were incubated for 1 hr at a room temperature, and the virus infectivity titer was estimated by agglutination of red blood cells in the wells.

3.4 The value, inverse of a common logarithm of the initial virus highest dilution, capable to induce positive hemagglutination reaction in a well, was assumed as the virus titer in control and experimental samples. The virus titer was expressed as the number of experimental infectivity doses (EID_{50}) or common logarithms EID_{50} per volume of the sample taken for titration. The virus titer was estimated in duplicates.

4. Coupon materials

Coupons from glass, fibrous cotton, and tile were used. Prior to the experiments, glass and tile coupons were cleaned with a sponge, washed with tap water, and sterilized. Fibrous cotton coupons were washed with tap water and sterilized in an autoclave.

5. Virus decontamination

5.1. Virus decontamination on coupons

5.1.1 0.2 ml of the infected allantoic fluid containing 10^7-10^8 EID_{50} was inoculated onto a sterile coupon surface with an automatic micropipette. Based on the initial virus titer and the contaminated coupon's square, the calculated infectivity dose for each of the samples was $5x10^6$-10^7 EID_{50}/cm^2.

5.1.2 The contaminated coupons were dried at RT and relative humidity 50-60% for 10 min till no liquid was observed on the coupons.

5.1.3 Then the coupons were positioned in the aerosol chamber 1.44 m^3 (50ft^3) on the floor, vertically on walls, and on the ceiling.

5.1.4 PAEROSOL was atomized with the VAG generator at the rate 5 ml/min to generate the aerosol with the drops size smaller than 10 μm and the drop mass median diameter d_{mmd} =3.0 μm. PAEROSOL was atomized for 1, 3, or 10 min. A continuously operating fan was positioned in the chamber for uniform aerosol distribution.

5.1.5 After each atomizing, the coupons remained inside the closed chamber for different time.

5.1.6 After the exposure, the coupons were withdrawn from the chamber, and the virus washed down with 10 ml of sterile physiological solution. The 0.5 ml samples were

taken for serial 10-fold dilutions from 10^{-1} to 10^{-9}. The virus infectivity titer was determined as described in the p. "Virus titration".

5.1.7 The virus inactivation was evaluated by a decrease of the virus titer on experimental compared to control coupons.

5.1.8 Prior to each subsequent PAEROSOL atomizing, the chamber was ventilated for 30 min to remove the aerosol remained.

5.1.9. To determine the initial virus titer, the samples were taken by washing virus down with sterile physiological solution just after drying of the contaminated coupons. The samples were titrated according to the procedure described in the p. "Virus titration". The determined virus titer was taken as the initial virus titer for each type of the coupons.

5.1.10. Three coupons were used per one measured point.

5.1.11. Statistical analysis was made with Microsoft-Excel Program. The average means of the virus titer as well as standard deviation were calculated.

RESULTS

1. Evaluation of the virus infectivity titer in dynamics after PAEROSOL application

Tables 1-3 demonstrate PAEROSOL decontamination efficacy toward viruses inoculated on glass coupons as the functions of PAEROSOL volume atomized (the time of EAS atomizing) and the time of exposure (coupons remained inside closed chamber after PAEROSOL atomizing). Glass coupons were positioned on the floor.

Table 1. PAEROSOL decontaminating efficacy toward avian flu A/NIBRG-14 (H5N1) virus on glass coupons as a function of the volume of atomized PAEROSOL (variable).

Volume of EAS atomized /50 ft^3.	0	5ml (1 min)	15 ml (3 min)	50 ml (10 min)
The virus titer $\log_{10}EID_{50}$(0.2ml)	7.0±0.0	4.3±0.5	2.5±0.5	0.2±0.4
Exposure time inside chamber after PAEROSOL distribution	10 min			

Table 2. PAEROSOL decontaminating efficacy toward avian flu A/NIBRG-14 (H5N1) virus on glass coupons as the function of a contact time (variable). Each time EAS was atomized during 3 min (15 mil/50ft^3), and then the coupons remained inside a chamber for pre-determined time.

Time the coupons remained inside a chamber after atomizing of PAEROSOL, min	0	5	20	60
The virus titer $\log_{10}EID_{50}$(0.2ml).	7.2±0.4	4.8±0.4	3.3±0.5	1.5±0.5
EAS atomized	15 mil/50ft^3			

Table 3. PAEROSOL decontaminating efficacy toward various viruses on glass coupons

Virus	EAS Atomized volume	Virus infectivity titer $\log_{10}EID_{50}$(0.2ml)		
		Prior to PAEROSOL atomizing	20 min after PAEROSOL atomizing	60 min after PAEROSOL atomizing
A/Puerto Rico/8/34 (H1N1)	15 ml/50 ft^3	6.5±0.5	3.2±0.4	0
A/NIBRG-14 (H5N1)		5.8±0.4	2.2±0.45	0.8±0.6
A/Duck/Kurgan/5/05 (H5N1)		5.7±0.5	2.3±0.5	1.0±0.3

As follows from Tables 1-3, PAEROSOL was equally effective to decontaminate three examined viruses. No reliable evidence of PAEROSOL efficacy dependence on virus serotype was observed. Additionally, the results presented in Tables 1-3 demonstrate that manipulating PAEROSOL volume and a contact time between viruses and PAEROSOL particles allowed increasing of decontaminating process effectiveness.

2. EAS aerosol decontamination of the viruses on different materials positioned inside the chamber.

The results of PAEROSOL decontamination efficacy toward the viruses inoculated on different materials are shown in Table 4. The experiment was conducted following the general protocol developed previously for *B. anthraces* simulate – the most hard-to-kill bio agent. This protocol was shown effective to eradicate spores and microbial cells on various materials.

Table 4. PAEROSOL efficacy toward avian flu virus A/NIBRG-14 (H5N1) on different materials positioned vertically and horizontally Viruses were inoculated on the coupons of different materials and positioned inside a chamber of 50 ft^3. 20 ml of EAS was atomized and the coupons remained inside the chamber during 12 hours.

Coupon location	Virus infectivity titer ($log_{10}EID_{50}$(0.2ml)					
	Glass		Tile		Fibrous cotton	
	Control	Experiment	Control	Experiment	Control	Experiment
	12 hours after 20 ml of phys. solution was atomized	12 hours after 20 ml of EAS was atomized	12 hours after 20 ml of phys solution was atomized	12 hours after 20 ml of EAS was atomized	12 hours after 20 ml of phys solution was atomized	12 hours after 20 ml of EAS was atomized
Floor	5.6±0.5	0	3.7±0.5	0	3.3±0,5	0
Wall		0		0		0
Ceiling		0		0		0

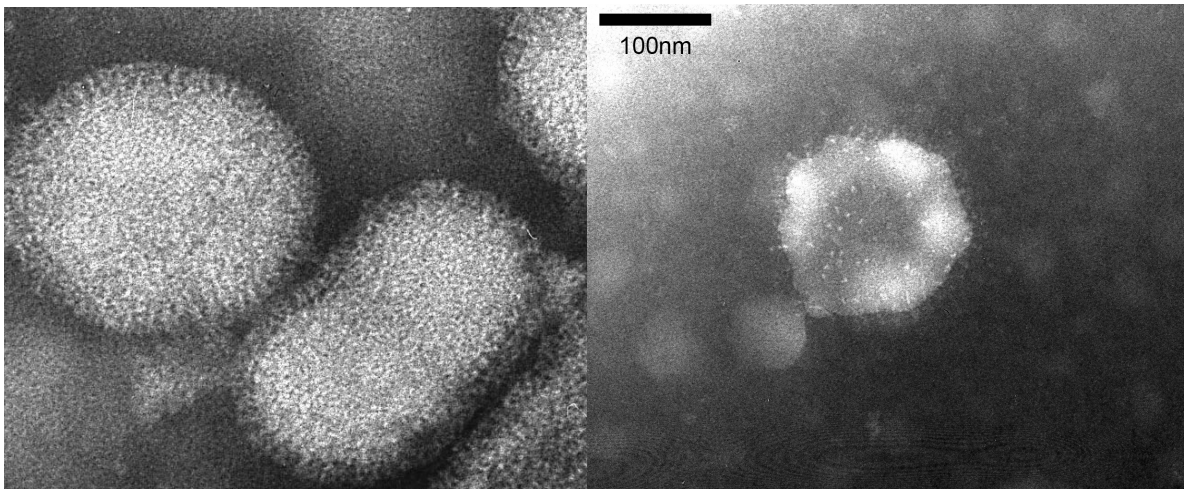
As evident from Table 4, the viruses were observed on the surfaces of all control coupons, which were subjected to the same conditions as experimental coupons except physiological solution was atomized instead of EAS. On cotton and tile coupons initial virus infectivity titers were lower (10^3 -10^4 EID_{50}/cm^2) compared to the calculated titer (10^5 EID_{50}/cm^2). This could be attributed to a few events simultaneously or in parallel occurred on the coupons. One of possible reasons could be poor virus recovery from certain surfaces like tile and fibrous cotton due to strong virus adsorption on these materials; it also could be caused by different virus inactivation on porous or fibrous surfaces during drying; it could be also the result of both reasons described above.

If only viral contamination is a target for decontamination, the chamber (or room) should remain closed for not longer than 60 min, or less. However, if general protocol is applied (12 hours the facility remains closed after PAEROSOL atomizing), high process effectiveness could be achieved in decontamination of all bioagents, including cells, spores, and viruses.

The results of this experiment also demonstrated that A/NIBRG-14 (H5N1) virus inoculated on different coupons (glass, tile, and fibrous cotton) was readily inactivated by PAEROSOL, and an efficacy was equal for the coupons positioned horizontally, vertically, and upside down.

3. Adding "light" to a mechanism of viruses' inactivation by PAEROSOL

Electron microscopic examination of the viruses subjected to a contact with PAEROSOL demonstrated that reduction of the virus infectivity titer was accompanied by the destruction of the virus surface, supposedly due to the destruction of two proteins - hemagglutinin and neuraminidase. These two proteins are known to be responsible for virus binding to a cell wall or virus budding. We have no enough data to speculate what exactly caused these proteins destruction, however the data presented in Fig 1 clearly demonstrate serious effect caused by PAEROSOL to virus surface. Fig 1 shows electron microphotographs of H5N1 virus: native virus prior experiment (a) and after virus contact with PAEROSOL (b).



A **B**

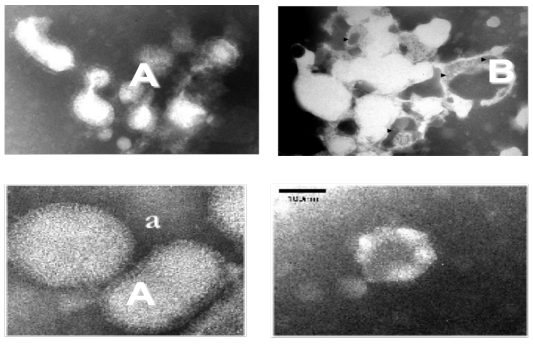


Fig 1. Virus A/Duck/Kurgan/5/05 (H5N1) morphology. Negative contrasting; x 50 000. Before PAEROSOL atomizing (left); After 3-5 min of the contact with PAEROSOL (right)

Conclusions

- PAEROSOL provides effective disinfection of surface-absorbed flu viruses
- PAEROSOL efficacy does not depend on virus serotype (at least for examined viruses)
- The coupons of different materials contaminated with viruses were decontaminated below detection level when 20 ml of PAEROSOL was atomized within the chamber of 50ft^3 and then the coupons remained in closed chamber overnight. The effectiveness of disinfection had no dependence on a coupon position inside a chamber.
- Electron microscopy demonstrated that deactivation of viruses caused by the contact with PAEROSOL was accompanied by the destruction of two virus surface proteins - hemagglutinin and neuraminidase.
- General Protocol, which was previously developed for disinfection of microbial cells and spores, was shown effective for decontamination of viruses on different materials.