Study of a Micro Aerosol of Electrochemically Activated Solution (PAEROSOL) against airborne viruses and viruses on different materials.

MATERIALS AND METHODS

1.Decontaminating agents

Electro-activated solution (EAS) was produced with STEL device (NPO "Ekran", Moscow). 20% (w/w) solution of NaCl in distilled water was processed through STEL along with distilled water at current intensity of 10-11 A (final concentration of NaCl ~1%). The content of active chlorine in EAS was 0.10 ± 0.01 % and pH 7.0 ± 0.2. EAS was used freshly prepared without sterilization.

Herein, PAEROSOL is atomized EAS

2. Viruses

Influenza virus A/Puerto Roco/8/34 (H1N1) from the depository of the Research Institute of Influenza RAMS (WHO Center of Influenza in RF) was used to inoculate the coupons and as airborne. The virus was cultivated in the allantoic fluid of 10-12-day chick embryos for 48 hrs at 36^0C, and the allantoic fluid was used for contamination.

3. Titration of virus

Virus titers in a control and in 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. The prepared dilutions were injected with a syringe to the allantoic cavities of chicken embryos (0.2 ml each), the opens sealed with paraffin, and the embryos incubated in a thermostat for 48 hrs at 36^0 C. 2 embryos were used per each virus dilution.

3.3. After termination of incubation, the embryos were chilled for a night at 4^0 C, shells were opened and the allantoic fluid samples were taken with a serological pipette. The sampled allantoic fluid was transferred to a titration plate (0.1 ml/well), and the equal volume of 1% suspension of chicken erythrocytes in physiological solution was added. The plates were incubated for 1 hr at a room temperature, and the virus infectivity titer was estimated by agglutination of erythrocytes in a well.

3.4. The value, inverse of a common logarithm of the original virus highest dilution, capable for induction of positive hemagglutination reaction in a well, was assumed as the virus titer in control and in the experiment. The virus titer was expressed as the number of experimental infectivity doses (EID_{50}) or as common logarithms EID_{50} per a volume of the sample taken for titration, similar to that described by Jakab et al. (1. Jakab GJ, Astry CL, Warr GA. Alveolitis induced by influenza virus. Am J Respir Dis 1983; 128:730-739).

4. Coupon materials

Coupons from glass (S=78.5 cm^2), fibrous cotton (S=25 cm^2), and tile (S=16 cm^2) 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 Inactivation

5.1. Virus inactivation on the coupons

For the experiments on virus inactivation, the allantoic fluid containing 10^7 -10^8 EID_{50}/0.2 ml of fluid was taken.

5.1.1. To contaminate coupons, the allantoic fluid was atomized with pneumatic atomizer (VMPS-MED, Fryazino, Moskovskaya reg.), which generated a coarse-dispersed aerosol. Based on the initial virus titer and the contaminated coupon's square, the infectivity dose for each of the samples was calculated. The volume of viral aerosol was 0.5 ml for glass coupon (the concentration of infectivity dose – 3.2 x 10^5 – 3.2 x 10^6 EID_{50}/cm^2) and 0.2 ml for fibrous cotton and tile coupons (the concentration of infectivity dose – 4 x10^5 – 4 x 10^6 EID_{50}/cm^2 and 6 x 10^5 – 6 x 10^6 EID_{50}/cm^2, respectively).

5.1.2. The coupons inoculated with virus were dried at T= (35±2) ˚ C and at relative humidity 50-60% for 30 min.

5.1.3. The coupons were positioned inside the aerosol chamber of 109.3 ft^3 (4.29 x 4.29 x 5.94 ft). The coupons were differently oriented inside the aerosol chamber - on the floor, vertically on the walls, and on the ceiling.

5.1.4. Liquid EAS was atomized for 1, 3, or 10 min with VAG-2 generator at the rate 5 ml/min to generate the PAEROSOL with droplet size less than 10 μm and a droplet mass

median diameter d_{mmd} =3 μm. A continuously operating fan was positioned in the chamber for uniform aerosol distribution.

5.1.5. After each aerosol generation, the chamber was tightly closed, and the coupons remained in the chamber for additional 30 min contact with EAS aerosol. A total time of EAS effect on the contaminated coupons consisted of the time for EAS atomizing and the time of contaminated coupons contacting with the EAS aerosol within the chamber.

5.1.6. Then the coupons were withdrawn from the chamber. Samples were taken by washing virus down with 10 ml of a sterile physiological solution, and the virus infectivity in each sample was determined by titration in chicken embryos. For this purpose, 0.5ml-samples were taken, subjected to serial dilutions from 10^{-1} to 10^{-9}, and the virus infectivity titer was determined as described above.

5.1.7. Prior to each subsequent PAEROSOL atomizing, the chamber was ventilated with a ventilator for 30 min to remove the aerosol remained.

5.1.8. Statistical analysis was made with the program Microsoft-Excel-insert-function-mean/standard deviation. The average meanings of the virus titer as well as standard deviation were calculated for each of the experimental groups.

5.1.9. A degree of influenza virus inactivation was evaluated by a decrease of the virus titer in the experiment compared to that in the control.

5.1.10 To determine the initial virus titer on the coupons, samples were taken by washing virus down with 10 ml of a sterile physiological solution just after drying of the virus containing allantoic fluid on coupons. The samples were titrated according to the procedure described in above. The determined virus titer was assumed as the initial virus titer for each of the coupon surface.

5.1.11. Three coupons were used per one measured point.

5.1.12. Three experiments were conducted in identical conditions

5.2. Decontamination of airborne viruses

For the experiments on inactivation of the airborne viruses, the allantoic fluid containing viruses in the concentration 10^7 -10^8 EID_{50}/0.2 ml of fluid was used.

5.2.1. To decontaminate airborne influenza viruses, the virus-containing allantoic fluid was centrifuged for 30 min at 4,000 rpm and T 4^0C. The obtained supernatant was centrifuged at 20,000 rpm and T 4^0C. To prepare the viruses suspension for atomizing, pellet

(viruses) was re-suspended in the volume of physiological solution equal to the initial volume of allantoic fluid to produce the virus suspension physically close to water.

5.2.2. 3.8 ml of the virus suspension containing 5 x 10^8 EID_{50}/ml was atomized inside the chamber with the pneumatic atomizer located in the centre of the chamber, to generate an aerosol at the calculated concentration 2.6 x 10^5 EID_{50}/ml with mass median diameter of drops d_{mmd} =2.5 μm).

5.2.3. PAEROSOL was atomized with the VAG-2 generator at the rate 5 ml/min (aerosol drops less than 10 μm and mass median diameter d_{mmd} =3 μm).

5.2.4. The control experiment was performed with physiological solution atomized instead of PAEROSOL.

5.2.5. Experimental protocol:

- Atomizing of virus-containing allantoic fluid inside the chamber - 1 minute.
- Pause - 1 minute (for uniform distribution of virus culture over the chamber; a fan positioned inside a chamber was turned on)
- Air sampling with a microcyclone devices to measure the concentration of airborne viruses in the chamber prior to EAS atomizing - 3 minutes
- Atomizing PAEROSOL (experiment), or physiological solution (control) with VAG-2 generator – 1 min
- Pause - 1 minute for uniform distribution of PAEROSOL (or MA of physiological solution) in the chamber (fan is on)
- Air sampling with a micro-cyclone device to measure the concentration of airborne viruses immediately after PAEROSOL (experiment) or MA of physiological solution (control) atomizing – 3 min.
- Pause - 2 minutes
- Next air sampling – 3 min
- Pause - 2 min.
- Next air sampling – 3 min.

5.2.6. Multiple air samples were taken from a chamber at certain intervals at the rate of 7 L/min. Micro-cyclone devices were positioned at 40 cm above chamber floor, on the front and sidewalls of the chamber. Each air sample was collected in 10 ml of physiological solution. A total contact time between PAEROSOL with airborne viruses was a time when

viruses remained inside a chamber (time prior each sampling). For instance, for first sample - PAEROSOL contact with airborne viruses began when PAEROSOL was atomized inside the chamber (p.5.2.5.), was continuing within a pause (1minute, p.5.2.5.) and was terminated with the completion of the first sampling (3 min., p.5.2.5), and so on. We proposed that a contact between PAEROSOL and viruses was terminated when a sample was collected in physiological solution. There, PAEROSOL were diluted by factor of~ 3,000.

5.2.7. In the samples sucked by micro-cyclones, the virus infectivity was determined according to the procedure described in p. "Virus titration".

RESULTS

Virus inactivation on coupons

Tables 1-7 represent experimental data on decontamination of airborne viruses and viruses inoculated on the coupons positioned inside the chamber of 109.3 ft^3. For the comparison, identical experiments were conducted with an aerosol of physiological solution instead of PAEROSOL.

Table 1. PAEROSOL efficacy with virus A/PR/8/34 (H1N1) inoculated on glass coupons.

Orientation of the coupons inside the chamber	Virus infectivity titer ((log_{10} EID_{50}/0.2 ml).)			
	Time of PAEROSOL atomizing at the rate 5ml/min*			
	0 min	1 min	3 min	10 min
Floor	3.8±0.69	1.3±0.94	0.0±0.00	0.0±0.00
Wall	2.7±0.47	0.0±0.00	0.0±0.00	0.0±0,.00
Ceiling	4.3±0,.47	0.8±0.69	0.0±0.00	0.0±0.00

*After atomizing of PAEROSOL, the coupons remained inside the chamber for additional 30 min contact with PAEROSOL

Table 2. Effect of a microaerosol of phys solution toward virus A/PR/8/34 (H1N1) on glass (control to the experiment presented in Table 1).

Orientation of the coupons inside the chamber	Virus infectivity titer ((log_{10} EID_{50}/0.2 ml)			
	Time of Phys solution atomizing at the rate 5ml/min*			
	0 min	1 min	3 min	10 min
Floor	3.8±0.90	3.8±0.69	4.0±0.58	3.3±0.47
Wall	3.7±0.47	2.9±0.47	4.0±0.75	3.3±0.47
Ceiling	4.5±0.50	4.3±0.75	3.8±0.69	4.0±0.58

*After atomizing of phys solution, the coupons remained inside the chamber for additional 30 min contact with Phys. solution aerosol

Table 3. PAEROSOL efficacy with virus A/PR/8/34 (H1N1) inoculated on tile

Orientation of the coupons inside the chamber	Virus infectivity titer ((log_{10} EID_{50}/0.2 ml).)			
	Time of PAEROSOL atomizing at the rate 5ml/min*			
	0 min	1 min	3 min	10 min
Floor	4.8±0,83	ND**	0.0±0,00	0.0±0,00
Wall	4,0±0,00	ND	0.0±0,00	0.0±0,00
Ceiling	4,3±0,47	ND	0.0±0,00	0.0±0,00

*After atomizing of PAEROSOL, the coupons remained inside the chamber for additional 30 min contact with PAEROSOL aerosol; **Omitted samples

Table 4. Effect of a microaerosol of phys solution toward virus A/PR/8/34 (H1N1) on tile (control to the experiment presented in Table 3).

Orientation of the coupons inside the chamber	Virus infectivity titer ((log_{10} EID_{50}/0.2 ml).)			
	Time of Phys solution atomizing at the rate 5ml/min*			
	0 min	1 min	3 min	10 min
Floor	4.2±0.69	ND**	4.2±0.90	3.8±0.69
Wall	3.8±0.69	ND	3,7±0.75	3.5±0.50
Ceiling	4.7±0.47	ND	4.3±0.75	4.3±0.47

*After phys solution atomizing, the coupons remained inside the chamber for additional 30 min contact with Phys. solution aerosol; **Omitted samples

Table 5. PAEROSOL efficacy with virus A/PR/8/34 (H1N1) inoculated on fibrous cotton coupons

Orientation of the coupons in the chamber	Virus infectivity titer ((log_{10} EID_{50}/0.2 ml))			
	Time of PAEROSOL atomizing at the rate 5ml/min*			
	0 min	1 min	3 min	10 min
Floor	3.8±0.37	ND*	0.0±0.00	0.0±0.00
Wall	3.5±0.50	ND	0.0±0.00	0.0±0.00
Ceiling	2.8±0.37	ND	0.0±0.00	0.0±0.00

*After PAEROSOL atomizing, the coupons remained inside the chamber for additional 30 min contact with EAS aerosol

**Omitted samples

Table 6. Effect of a microaerosol of phys solution toward virus A/PR/8/34 (H1N1) on fibrous cotton (control to the experiment presented in Table 5).

Orientation of the coupons inside the chamber	Virus infectivity titer (log_{10} EID_{50}/0.2 ml).)			
	Time of Phys solution atomizing at the rate 5ml/min*			
	0 min	1 min	3 min	10 min
Floor	4.2±0,37	ND *	4.2±0,69	4.3±0.47
Wall	3.7±0,47	ND	3.5±0,75	3.3±0.47
Потолок	3.5±0,50	ND	3.7±0,47	3.3±0.47

*After phys solution atomizing, the coupons remained inside the chamber for additional 30 min contact with Phys. solution aerosol. **Omitted samples

It was shown that one minute of EAS atomizing (5 ml of PAEROSOL per 109ft^3) followed by additional 30 min of the coupons contact with aerosol in the airtight chamber, was not enough to completely inactivate influenza virus on selected coupons (Tables 1). However, 15 ml of PAEROSOL atomized during 3 minutes followed by additional 30 min of the coupons contact with aerosol in the airtight chamber, resulted in a decrease of virus infectivity on the coupons to the value below detection limit on all tested materials.

It was previously demonstrated (Reports 2.2.1 and 2.2.2) that decontamination of spores and microbial cells required significantly longer time and larger volume of PAEROSOL, than it was required for viruses. Therefore, we proposed that the protocol developed for vegetative cells' and spores' decontamination would be effective for the viruses decon as well.

Inactivation of the airborne virus

To study PAEROSOL efficacy toward airborne influenza virus, virus particles were additionally purified from the components of allantoic fluid (see Mat & Meth). It was demonstrated that sedimentation of the influenza virus from allantoic fluid followed by re-suspending of viruses in physiological solution did not affect the virus infectivity. Table 7 demonstrate PAEROSOL efficacy toward airborne viruses.

Table 7. PAEROSOL efficacy with airborne virus A/PR/8/34 (H1N1)
(PAEROSOL was atomized during 1 min; 5ml of PAEROSOL was dispersed inside a chamber of 109ft3

Airborne virus infectivity titer (log_{10} EID_{50}/0.2 ml) after the contact with PAEROSOL*			
Time after PAEROSOL atomizing			
0 min	5 min	10 min	15 min
3.5±0.50	0.0±0.00	0.0±000	0.0±0.00
Airborne virus infectivity titer (log_{10} EID_{50}/0.2 ml) after the contact with an aerosol of physiological solution **			
Time after Phys solution atomizing			
0 min	5 min	10 min	15 min
3.5±0.50	3.3±0.47	3.0±0.58	3.3±0.47

*Total time of EAS aerosol contact with viruses consisted of the time for EAS atomizing (1 min at the rate 5 ml/min) and the time of contacting with EAS aerosol while virus remaining in the chamber.
** Total time of phys solution' aerosol with influenza virus consisted of the time for phys. solution atomizing (1 min at the rate 5 ml/min) and the time of contacting with phys. solution aerosol while virus remained within the chamber.

As followed from Table 7, virus titer was reduced below detection level after 5 min of virus contact with EAS aerosol though no virus inactivation was caused by a contact with the aerosol of physiological solution. It should be noted that airborne virus titer was proposed (calculated) as 2.6 x 10^5 $\log_{10}EID_{50}$/0.2 ml, or 6.7 x 10^8 EID_{50}/m^3. However, the results demonstrated noticeable difference between proposed and real concentration of airborne viruses. Instead of proposed 6.7 x 10^8 EID_{50}/m^3, measured values where in the range 3 x 10^6 - 4 x 10^7 EID_{50}/m^3. No explanation for a difference between proposed and measured concentrations of airborne viruses is available at the moment.

CONCLUSIONS

1. PAEROSOL efficacy to decontaminate influenza virus on selected materials was demonstrated. 3 min of PAEROSOL atomizing at the rate 5 ml/min followed by 30 min of the coupons exposure inside the chamber resulted in complete deactivation of the viruses
2. 5 ml of PAEROSOL dispersed inside a chamber of 109ft3 and 5 min of airborne virus contact with PAEROSOL, reduced airborne viruses below detection limit.