TEST REPORT

WITH TEQOYA IONIZER

SCOPE OF WORK

Effect of ionization on nebulized droplets containing Influenza A/H1N1/ PR8 virus

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SAPIENZA
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Test report

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CONTEXT AND EXPERIMENT SET UP

Emersum company and University of Sapienza defined a protocol with the aim to evaluate the **effect on the Influenza A/H1N1/PR8 virus in air**.
This protocol was based on several scientific paper of reference and defined indipendently from TEQOYA.
The model analyzed to test ionization is the Teqoya TIP4 due to constraints of biosafety cabinet employed in microbiology to test the virus.

Private bodies involved: Partner and promoter of the research:

- Dr. Daniele Biscontini. Chief Technology Officer of Emersum s.r.l., SIAVS, Air Quality Specialist & Innovator www.emersum.eu;

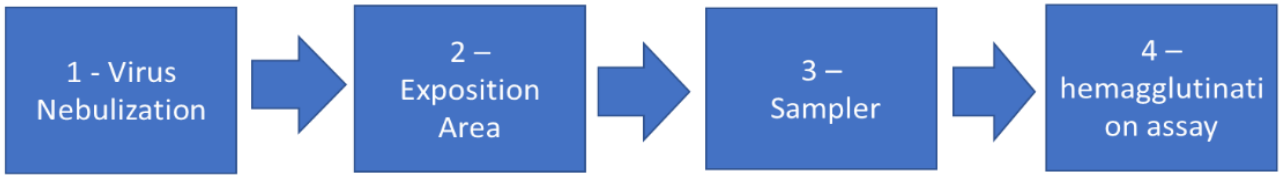
Public research and medical institutions of excellence:

- Research coordinator: Prof. Dr. Marcello Vitale, Environmental Modelling Laboratory, Department of Environmental Biology of the "Sapienza" University of Rome;
- Research collaboration: Prof. Anna Teresa Palamara, Professor of Microbiology, Department of Public Health and Infectious Diseases of the "Sapienza" University of Rome.

Air purifier Company and Model evaluated:

- Company: TEQOYA SAS, 7, route de Préchac - 33730 Villandraut - France;
- Model: Teqoya TIP4;

GENERAL PROTOCOL SCHEME



LIST OF MATERIALS

The experimental system, showed above and described below, was included in a certified class II biosafety cabinet (S@femate EZ 1.2 Euroclone):

1. Droplet nebulizer:
    a. nebulizer with 4 L/min brand input flow and some components are shown in Figure 1. The average size of droplets was 2.5 μm. Mass median aerodynamic diameter was 2.95 μm. Fine Particle Profile: Breathing Fraction <5 μm: 74.7%. Nebulizer flow: 0.18 mL/min.
    b. Mist liquid: 1 mL of Roswell Park Memorial Institute (RPMI) 1640 Medium containing 10^{11} plaque-forming units per ml (PFU/mL) of H1N1 virus, 8 mL of deionized water, and 0.05 mL of PBS (Phosphate Buffered Saline) Solution.
    c. Air dryer: Wilkerson X06-02-02 used for controlling Relative Humidity (RH) values;

2. Ionization exposure cube: the ionizer **Teqoya TIP4 model** was placed inside a polycarbonate cube of 40 × 25 × 25 cm^3, the power of which can be managed remotely through Bluetooth remote management system for switching on and off of all devices;
    a. Particle size Sensor: 2.5 μm particle for 0.1L of analysed air, in order to check for the presence of droplets with appropriate size;
    b. Relative Humidity and Temperature sensor:

3. Sampler: SKC biosampler at 12.5 L/min flow;

4. Hemagglutination assay (HA):
    a. Hemagglutination test using chicken red blood cells diluted in PBS.
    b. Mathematical evaluation of the ionization effect:
    Dilution factor = HA Pre/HA Post,
    Density reduction = 1 – (HA Post/HA Pre).

Emersum has built a system for analysing the performance of air quality instruments. Unlike normal air quality performance analysis systems, this instrument has not previously been fumigated with aerosol and, then, the extent of the particle abatement was subsequently analysed.

Experimental conditions foreseen a continuous nebulization flow of droplets containing the influenza H1N1 virus. Simultaneously to nebulization, a controlled flow of dried air was fluxed inside, in order to monitor and check internal relative humidity. Furthermore, a continuous air mixer, constituted by a plastic fan, was placed inside the experimental cube. Finally, a pump continuously sucked the air containing the influenza virus into a biosampler, where it condensed in contact with a known volume solution placed inside it.

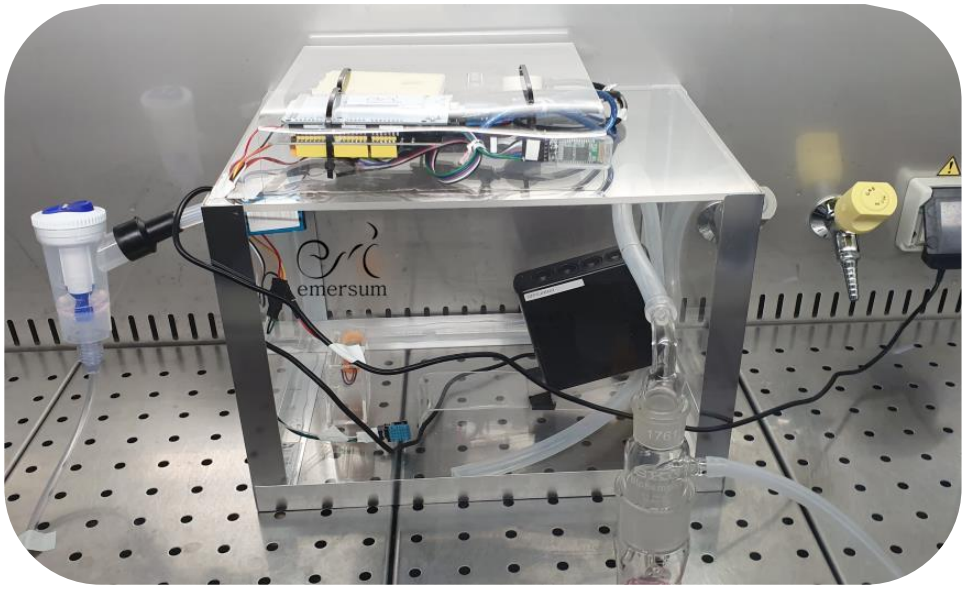


Figure 1 – Experimental system: Nebulization (Left), exposition (centre), biosampler collector (bottom)

METHODS

The following section describes the methodologies used for evaluation of ionization effect on nebulized viral particles.

Experimental settings: in order to collect a sufficient amount of influenza virus, the experimental system operated in two cycles of 25 minutes each, where 8mL of solution containing virus was nebulized into the cube per cycle. A control cycle with the ionizer switched off was run.
The particulate matter (PM) counter sensor, placed inside the experimental cube, showed a constant particle concentration of 2500 $\mu g/m^3$ of $PM_{2.5}$ during the experimental runs. It is important to note that this PM concentration must be considered to be very high, corresponding to extreme experimental conditions with respect to environmental PM values.

A minimum exposure of mixed air to ions generated by ionizer was set at 3 second, whereas a maximum of 20 seconds was considered for the inlet flow.

Three conditions were tested in the General Protocol Scheme:

- Pre and After nebulization, without control of RH, then with a stable RH at 90%, virus titer diluted 1:100;
- Pre and After nebulization, without control of RH, then with a stable RH at 90%, virus undiluted;
- Pre and After nebulization, with controlled of RH, stable RH at 56%, virus undiluted;

Cross-check test:

- After nebulization all internal surfaces of the cube chamber were sampled with cotton swab and dissolved in RPMI medium to eventually collect viral particles on the surfaces of the cube (Figure 4);
- Two 35mm cell-culture dishes containing 1mL of RPMI were placed into the experimental cube to evaluate a possible viral settling down on the cube floor (Figure 5);

Hemagglutination assay

The hemagglutination assay was performed to evaluate the effect of ionization on the viral particles. Indeed, the assay is based on the capability of the hemagglutining protein (HA), exposed on the viral envelope, to agglutinate chicken red blood cells (Figure 3a-b). This assay is usually used to estimate the amount of viral particles (hemagglutinin unit, HAU) in a solution and, in this case, it can evaluate the potential inactivation of viral envelope after ionization. The test was performed on the pre-nebulized and post-nebulized (biosampler) solutions in the presence and in the absence of controlled ionization.

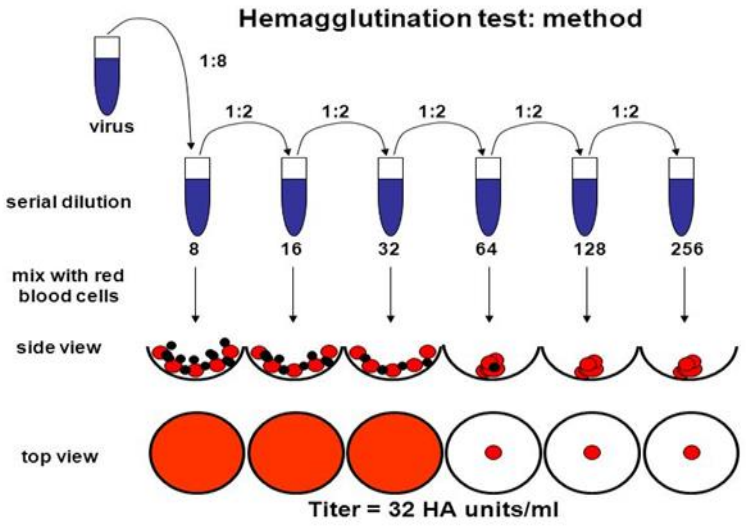


Figure 2a – Schematic representation of hemagglutination test

RESULTS

After three test cycles carried out at different initial virus concentrations and different relative humidity values, controlled ionization reduced the viral titer, measured by HAU, of approximately 75% (from 128 to 32 HAU, and from 256 to 64 HAU) respect to the non-ionized control.

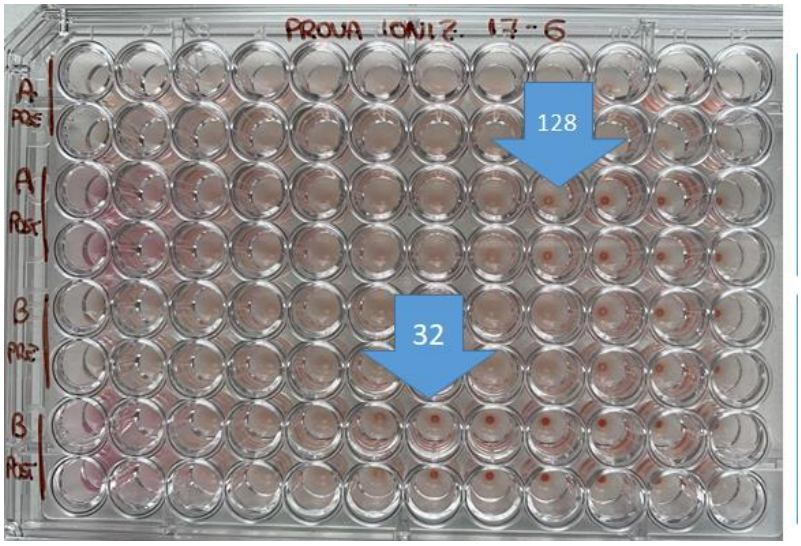


Figure 3b –Representative image of hemagglutination results

Table 1 – environmental parameters, initial virus concentrations and HAU values measured in the pre-nebulize and post-nebulized solutions in the presence and in the absence of ionization.

Viral titer	Avg. temp	Avg. RH	Pre Nebul. Control: Ionizer OFF	POST Nebul. Control: Ionizer OFF	Pre Nebul. Test: Ionizer ON	POST Nebul. Test: Ionizer ON	Dilution factor	Density reduction
1.3×10^{11} PFU (diluted 1:100)	27°C	90%	32	**4**	16	**0**	**4**	**75-100%**
1.3×10^{11} PFU (undiluted)	27°C	90%	256	**128**	256	**32**	**4**	**75%**
1.3×10^{11} PFU (undiluted)	28°C	56%	512	**256**	512	**64**	**4**	**75%**

Cross-check test

To check for the possible viral settling down on the surfaces of the cube, sampling of internal surfaces using a cotton swab after each run and placing two 35mm cell-culture dishes containing 1ml of RPMI during each run, were performed. Both sampling failed in detecting any HAU.

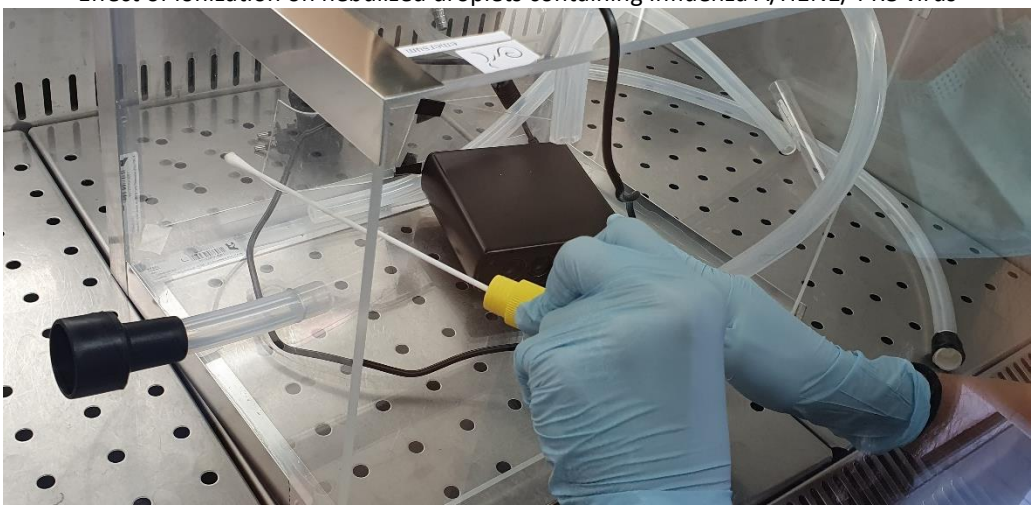


Figure 4 – Internal cube surfaces sampling by swabbing

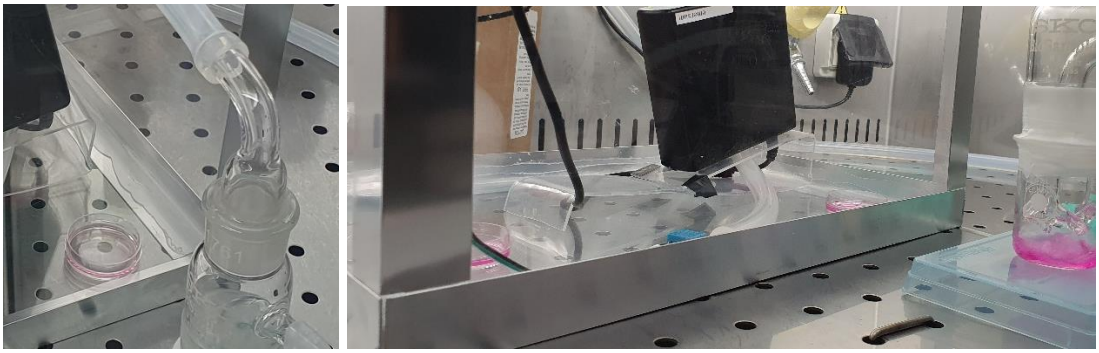


Figure 5 – Positioning of two 35mm cell-culture dishes containing 1 mL of RMPI to evaluate possible viral settling down during nebulization and ionization

Table 2 – environmental parameters, initial virus concentrations and HAU values measured in the pre-nebulized and post-nebulized solutions in the presence and in the absence of ionization.

Test	Avg. temp	Avg. RH	POST Nebul. Control: Ionizer OFF	POST Nebul. Test: Ionizer ON	Dilution factor	Density reduction
Cotton swab	28°C	56%	**0**	**0**	**0**	**0**
35mm dishes	28°C	56%	**0**	**0**	**0**	**0**

CONCLUSIONS

The results of the experiments demonstrated a reduction of HAU of approximately 75%, in the sampling runs undergone to controlled ionization, thus suggesting a decrease in droplets containing virus.

The observed reduction in HAUs could be explained by the following hypotheses:

A. the virus was damaged at the envelope structure level;
B. the virus was completely destroyed;
C. the viral particles were adhering to the cube surfaces and they could not be collected in the biosampler.

To evaluate the C hypothesis a Cross-check test was performed by sampling internal surfaces of the cube. Because no HAUs were obtained by cross-check test, the following hypotheses are suggested:

- an insufficient amount of recovered viral particles for HAU detection;
- an absence of adherent viral particles on the internal cube surfaces;
- adherent viral particles were structurally altered or destroyed.

In conclusion, an active role of the controlled ionisation on reduction of viral particles of the influenza virus H1N1 has been demonstrated.

Further analyses and experimental trials should be carried out in order to also characterise the reduction of infective capacity of the virus and/or its structural and functional destruction in airborne droplets and on surfaces.

The scientific responsible and coordinator of the experimental trials

Prof. Marcello Vitale