YONSEI UNIVERSITY

Final Report

Project name: A study of the inactivation of airborne influenza viruses by an ion generator produced by Samsung Electronics

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1. Purpose and background of study

Many people throughout the world are admitted to hospitals every year due to high fever and respiratory disease caused by influenza, which has an especially high mortality rate in children and elderly persons. Also, influenza can result in higher mortality rates when accompanied with respiratory complications such as pneumonia and other cardiopulmonary diseases. In addition to such effects on national healthcare, influenza can also have negative effects on the national economy by causing losses in labor due to hospitalization and clinic visits. According to the Jordan Report 2000, the number of deaths caused by disease related to influenza reaches 20 thousand per year in the US alone. This can spike up to 50 thousand in cases with the prevalence of more severe influenzas. While the lives lost due to influenza are important, what is also important is the expense required to treat patients along with the economic cost due to time lost in studies and work that according to some reports, can reach tens of billions of dollars per year. Therefore, the benefits that can be obtained by preventing influenza can be considered to be enormously large.

Every year, new species of the influenza virus prevail due to mutation of the viral antibody. Such mutation of the antibody differs considering the sensitivity and infectivity of the host and sometimes can cause serious pandemics. The influenza pandemic of 1918-1919 killed 20 to 40 million people worldwide, and according to some reports, 196 thousand people died in the US alone in just one month: October 1918. Also, there were pandemics in 1957, 1968 and 1977 which resulted in the deaths of hundreds of thousands of people. In 1997, the Hong Kong H5N1 influenza was reported as the first avian influenza directly infectious to humans, and as of present in 2005, the world is facing difficulties in controlling influenza as there are still patients dying due to avian influenza in countries such as Vietnam. As described above, influenza has a long history of causing deaths and economic losses. However, at the present moment, the world does not have an effective way of inhibiting the disease. Though there are some vaccines and treatments that are currently being used, the treatments are not able to provide perfect prevention and cure. And though the development of vaccines and treatments is important, prevention of viral infections in an environmental aspect is also fundamentally important. If we can reduce or eliminate airborne influenza viruses mechanically, it will reduce the opportunity of infection which will provide a great assistance in preventing influenza.

This study intends to verify the effect of the ion generator developed by Samsung Electronics in eliminating influenza viruses.

2. Equipments and materials

2.1 Equipments and materials

2.1.1 Chamber (1 m^3)

2.1.2 Air sampler (MAS-100eco of Merck)

2.1.3 Spray gun

2.1.4 Biosafety cabinet

2.1.5 CO2 incubator

2.1.6 Ultra-cold refrigerator (-80°C)

2.1.7 Water bath

2.1.8 Refrigerator

2.1.9 Incubator

2.1.10 Ion generator (Samsung Electronics)

2.1.11 High pressure sterilizer

2.1.12 Conical tube (15ml, 50ml)

2.1.13 Silica gel

2.1.14 Thermometer and hygrometer

2.1.15 Pipet aid

2.1.16 Plastic pipet (10ml)

2.1.17 Microcentrifuge tube (1.5ml)

2.1.18 Multi-well plate (6 well)

2.1.19 T75 culture plate

2.1.20 Syringe (10ml)

2.1.21 Syringe filter (0.2μm)

2.1.22 Petri dish

2.2 Materials to be tested

2.1.1 Influenza A virus (attenuated type)

2.1.2 MDCK animal cell line

2.1.3 MEM (animal cell culture media)

2.1.4 Embryonated eggs (virus culture)

2.1.5 PBS

2.1.6 Trypsin

2.1.7 Crystal violet

2.1.8 Formaldehyde solution

2.1.9 ethyl alcohol

2.1.10 Low melting agarose

2.1.11 Fixed chicken red blood cell

3. Test method

3.1 Preparation of sample (virus culture)

After culturing embryonated eggs for 11 days at 37°C, only embryonated eggs with normal growth were selected through egg inspection. After inoculation of a certain amount of virus in the embryonated egg, the eggs were cultured in an incubator again for 3-4 days. The air sacs of the embryonated eggs were then cut out and the allantoic fluid including the virus was harvested. Through the hemagglutination assay (HA assay), the amount of the virus was measured and filtered with a syringe filter and stored at -80°C.

3.2 Inoculation of test sample (spraying of virus)

11ml of the virus solution was put in a spray gun and sprayed equally using the pressure of compressed air.

3.3 Sampling

- The first sampling was done 10 minutes after the virus was sprayed. Samples of 50L were taken using an air sampler (MAS-100eco) from Merck. Sampling at 20 minutes after the virus was sprayed was conducted by taking 150L and 400L for sampling at 40 minutes and 1000L for sampling at 1 hour. MEM 3ml was used as the culture medium for sampling.
- Immediately after the first sampling, the ion generator was activated and sampling was done 20 minutes after activation, and sampling was done for up to 1 hour at 20 minute intervals (0, 2, 20, 40, 60min).
- Testing was conducted twice each with once for control with the ion generator inactive, and once with the generator active.
- The temperature of the chamber was maintained closely at 25°C without exceeding by more than 1°C from 25°C.
- 2 mini fans were used in order to circulate the air.
- The blow fan was used only during spraying.

3.4 Plaque assay (culture exam for residual virus)

- The MDK cell line was cultured up to a confluent state in a multi-well plate (6 well).
- The sample was melted and 10 fold serial dilution was conducted for initial samples and was inoculated to the MDCK cell for 1 hour.
- The amount of the samples used for inoculation was 500μL.
- After inoculation, 2x MEM media and low melting agarose were mixed equally and poured on the cell and hardened.
- Incubation was done in the CO2 incubator for 3-4 days and dyed with crystal violet so that the plaque emerged by the virus could be easily observed.

3.5 Description of results

- The results were described as averages of the 2 trials.
- The reduction rate and residual rate were calculated with the following formulas.

Reduction rate (%) = $\frac{B}{A}$ x 100

Residual rate (%) = 100 – reduction rate

A: Number of viruses of sample first collected before the ion generator is generated

B: Number of viruses of sample collected at each time after the ion generator is generated

4. Study results

The testing of the ion generator's effects in eliminating influenza virus was conducted twice each. Tests were conducted for control (sampling when ion generator was not activated, natural reduction) and when the ion generator was activated, and collection of samples was done 10 minutes after virus sampling. Then samples were collected at 20-minute intervals and tested up to 1 hour. The results of the elimination effects for influenza virus at the temperature and humidity described above are shown below.

4.1 Average temperature and humidity condition

- Temperature: 25±1°C
- Humidity: The relative humidity was at first high in the early phase as a large amount of the virus solution was sprayed in a closed chamber, and then gradually decreased due to sampling and a rise in temperature. The humidity was 60±2% when the virus was first sprayed and maintained 51±3% after 1 hour.

4.2 Test results

Table 1. Number of viruses of control and when ion generator was activated at each time (log10)

	0 time	20min	40min	60min
Control	5.31	4.93	4.38	4.23
Ion	5.31	3.85	2.86	1.85

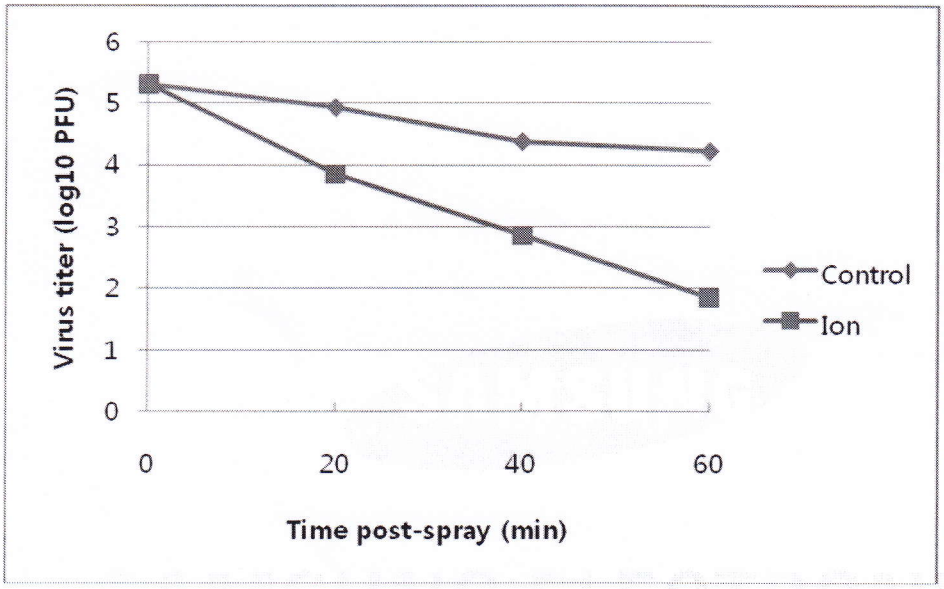

Figure 1. Graph comparing the average of the test results

Table 2. Residual rate of the influenza virus of each time of the activation of the ion generator (%)

	0 time	20min	40min	60min
Control	100	100	100	100
Ion	100	8.1	3	0.4

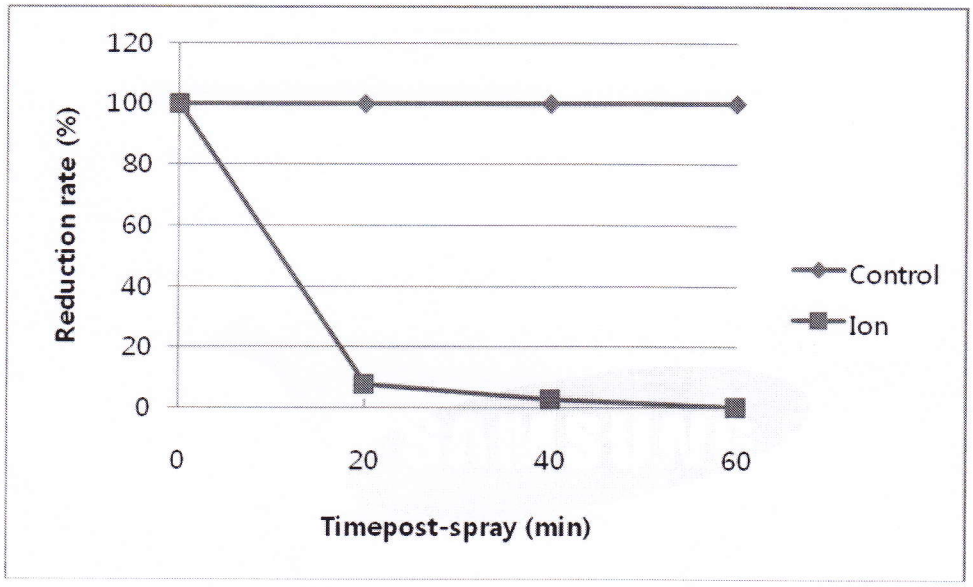

Figure 2. Graph showing the residual rate tested with the ion generator

Table 3. Reduction rate of the influenza virus of each time of activation of the ion generator (%)

	0 time	20min	40min	60min
Control	0	0	0	0
Ion	0	91.9	97	99.6

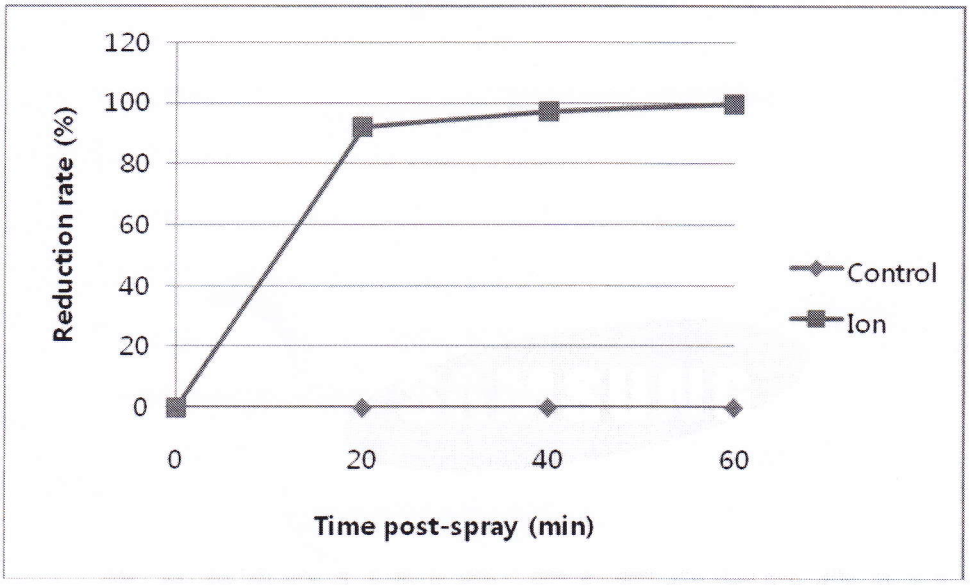

Figure 3. Graph showing the reduction rate tested with the ion generator

5. Conclusion

Putting together the above results, we can see that the ion generator of Samsung Electronics can reduce the influenza virus by more than 99% within 1 hour.

The results of this study were based on tests conducted in an approximately 1000L space at 25°C and at a relative humidity of 50-60%. The actual functionality of the tested device can differ in household environments where temperature, humidity and spatial size are different.