Research Report

Inactivation of Major Airborne Viruses by the Viruskiller

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Ⅰ. Introduction

It is very important for human beings not to be infected from various microbes in the air where infectious microbes could be present in living environments. Of many kinds of microbes in air, viruses particularly can cause severe diseases in human. Generally viruses can be present longer time than other microbes in aerosols and some viruses cause critical diseases as causative agents of secondary infections in immunocompromised patients and old persons.

Considering the importance of viruses in air environments, the INB Ltd developed and manufactured the Viruskiller. The Viruskiller is supposed to inactivate all kinds of viruses present in various environments.

In the present study, we tried to demonstrate the ability of the Viruskiller to inactivate viruses which might be present in air environment. Three representative airborne viruses were selected for the test. Then those viruses were added to the Viruskiller and infectiveness of the viruses contained in the exit air were examined using corresponding permissive cell lines. Poliovirus was selected for their rigidity and comparative long survival as a representative virus of many species of picornavirus. Adenovirus was selected for its causative agent of many kinds of infectious diseases including eye and alimentary tract. Influenza virus was selected for its severity and unique characteristics as large enveloped RNA virus.

It is considered that the Viruskiller can inactivate most viruses present in air when the above three viruses are successfully inactivated during the airflow through the Viruskiller.

We report the test results on the inactivation of 3 viruses by the Viruskiller manufactured by the INB Ltd.

Ⅱ. Materials and Methods

1) Materials

(1) Viruses and their potencies

Poliovirus type III was distributed by Korean National Institute of Environment Research and used for the test. Its potency for the test was 10^6 Plaque Forming Unit(PFU)/100 ㎖ phosphate buffered saline(PBS). Influenza virus (A/Johannesburg, H3N2) was distributed by Korean National of Health and used for the test. Its potency was 10^6 $TCID_{50}$/100 ㎖ PBS. Adenovirus type II was distributed by Korean National Institute of Health and its potency for the test was 10^6 $TCID_{50}$ /100 ㎖ PBS.

(2) Cell lines and their culture

BGM cell line was used for the growth of poliovirus and cultured in DMEM media. 10% of calf serum was added for growth medium and one % of foetal calf serum for the maintenance of the cell and virus culture. MDCK cell line was used for the growth of influenza virus. Adenovirus was cultured using A549 cell lines.

The above 3 cell lines were cultured in 5% CO_2 incubator. The viruses were added to 80% monolayer sheets 2 or 3 days after cell passages.

(3) The Viruskiller

The Viruskiller was developed and manufactured by INB Ltd. One of the major functions of the Viruskiller was designed to inactivate viruses in air.

2) Methods

(1) Inoculation of viruses into the Viruskiller

The amount of 10^6 PFU viruses was added to 100 ㎖ PBS. Whole the PBS solution was sprayed into the Viruskiller as shown in figure 1. The air was flowed through the Viruskiller and exit air was flowed through the MDS filter paper attached at the exit of the machine as shown in figure 2.

Figure 1). Virus was sprayed into the duct of Viruskiller

Figure 2). MDS filter attacked at the exit of the Viruskiller

(2) Elution and concentration of the attached virus

The MDS filter paper was adsorbed in pH 9.5 beef extract solution for the elution of virus attached in the filter paper. The pH of the beef extract solution was adjusted to 7.0 using 1 M HCl. Then pH of the solution was lowered to 3.5 and kept for 30 minutes at room temperature. The precipitate was taken after centrifugation at 2500xg for 15 minutes. The precipitate was solved with pH 9.2 PBS at room temperature for 10 minutes.

Then the solution was centrifuged at 9,000xg for 10 minutes at 4℃. The supernatant from the solution was taken and acidity was adjusted to pH 7.2. The solutions were used to inoculate into the corresponding cells for infectious virus culture after filtration with 0.22 ㎛ millipore filter.

(3) Inoculation of the specimen into cell lines

The final concentrate specimen of 0.75 ㎖ was inoculated into 20 monolayered cell culture flasks of corresponding cell lines. Inoculated cells were incubated for 100 minutes for adsorption of the virus into cells.

(4) Observation of cytopathic effect by infectious viruses

Specific cytopathic effects on inoculated cell sheets were observed for 14 days after inoculation. Multiplication of viruses were read by cytopathic effect on cells using inverted microscope.

III. Results

The presence of infectious virus from the exit air flowed through the Viruskiller was tested using corresponding permissive cell lines of viruses.

First, concentrate from 10^6 PFU polio type 3 in 100 ㎖ PBS flowed through the Viruskiller was inoculated into BGM cells for the virus culture. The BGM cells inoculated with specimen showed no cytopathic effect as shown in figure 3. On the contrary, BGM cells inoculated with poliovirus type 3 treated with same procedures as specimen flowed through the Viruskiller showed typical poliovirus specific CPE as shown in figure 4.

Second, concentrate from 10^6 $TCID_{50}$ influenza virus in 100 ㎖ PBS flowed through the Viruskiller was inoculated into MDCK cells for the virus culture. The MDCK cells inoculated with specimen showed no cytopathic effect as shown in figure 5. On the contrary, MDCK cells inoculated with influenza virus treated with same procedures as specimen flowed through the Viruskiller showed typical influenza virus specific CPE as shown in figure 6.

Third, concentrate from 10^6 $TCID_{50}$ adenovirus type 2 in 100 ㎖ PBS flowed through the Viruskiller was inoculated into A549 cells for the virus culture. The A549 cells inoculated with specimen showed no cytopathic effect as shown in figure 7. On the contrary, A549 cells inoculated with Adenovirususus type 2 treated with same procedures as specimen flowed through the Viruskiller showed typical adenovirus specific CPE as shown in figure 8.

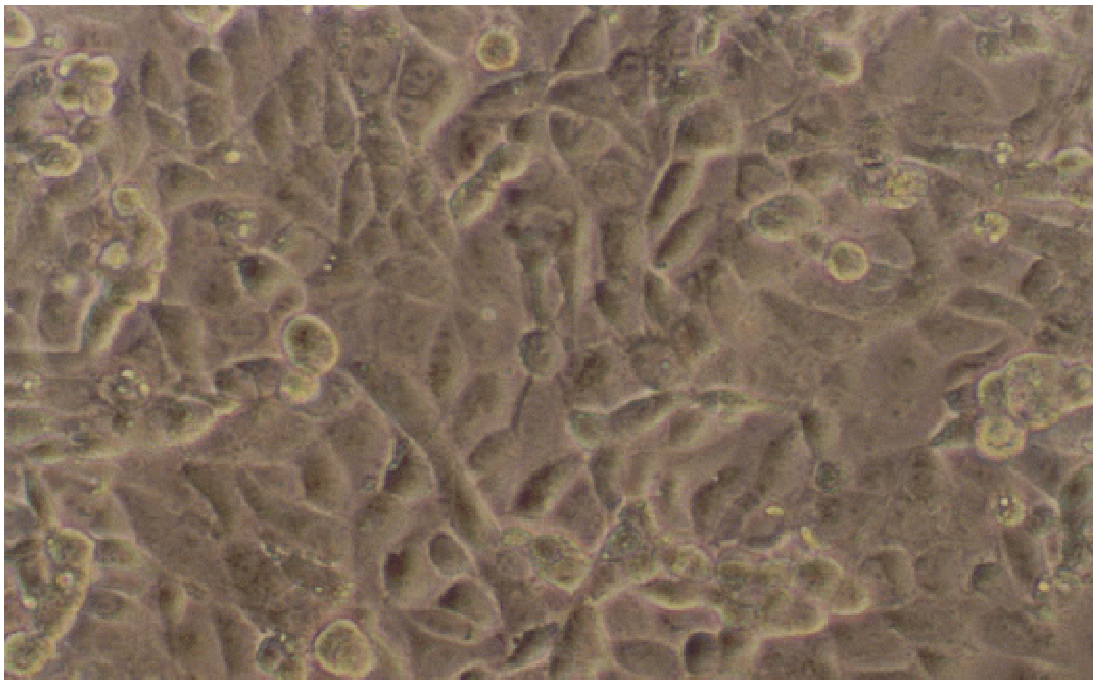

Figure 3). BGM cells inoculated with Polio virus concentrate from the Viruskiller, ×200

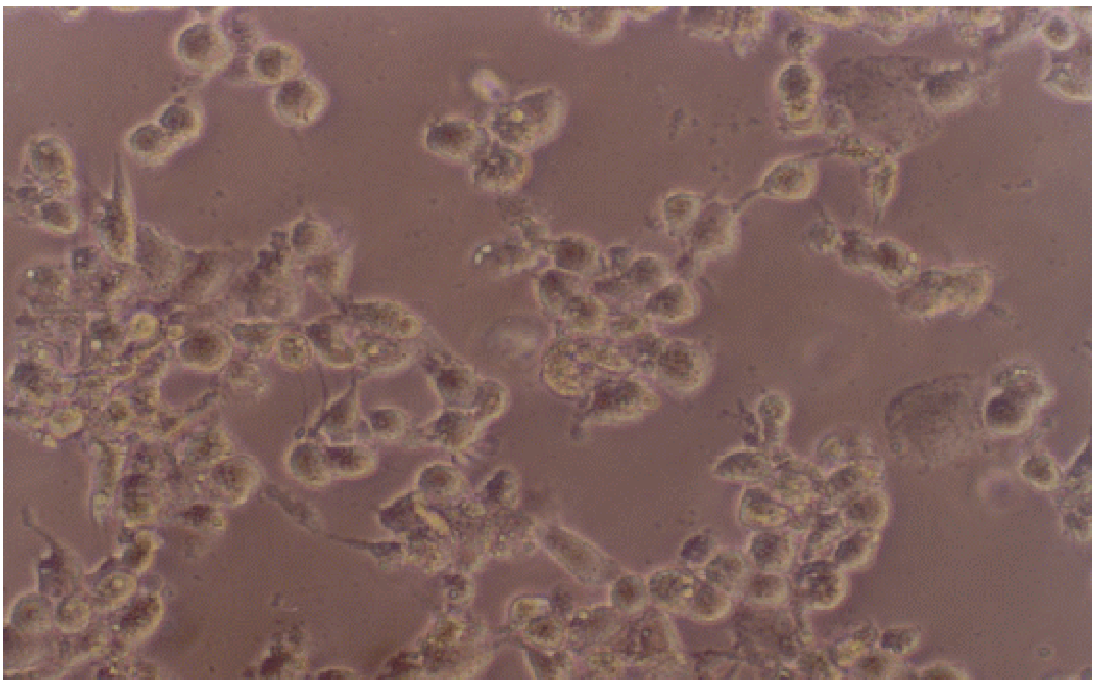

Figure 4). Polio virus type 3 specific CPE shown on BGM cells, (Positive, ×200)

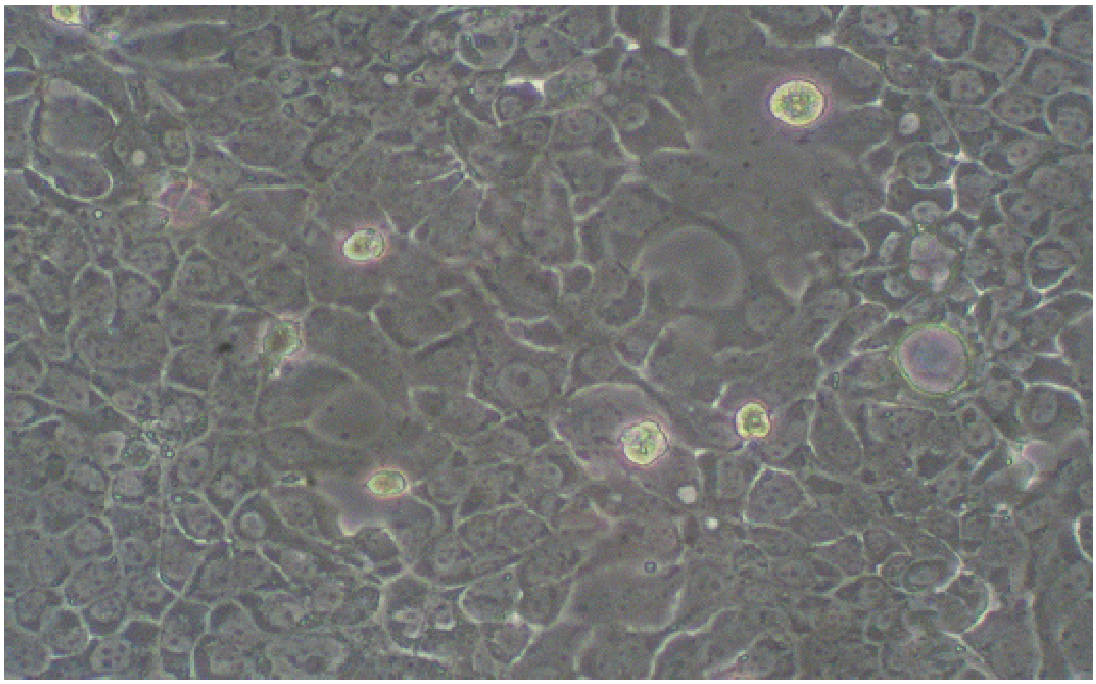

Figure 5). MDCK cells inoculated with Influenza virus concentrate from the Viruskiller, ×200

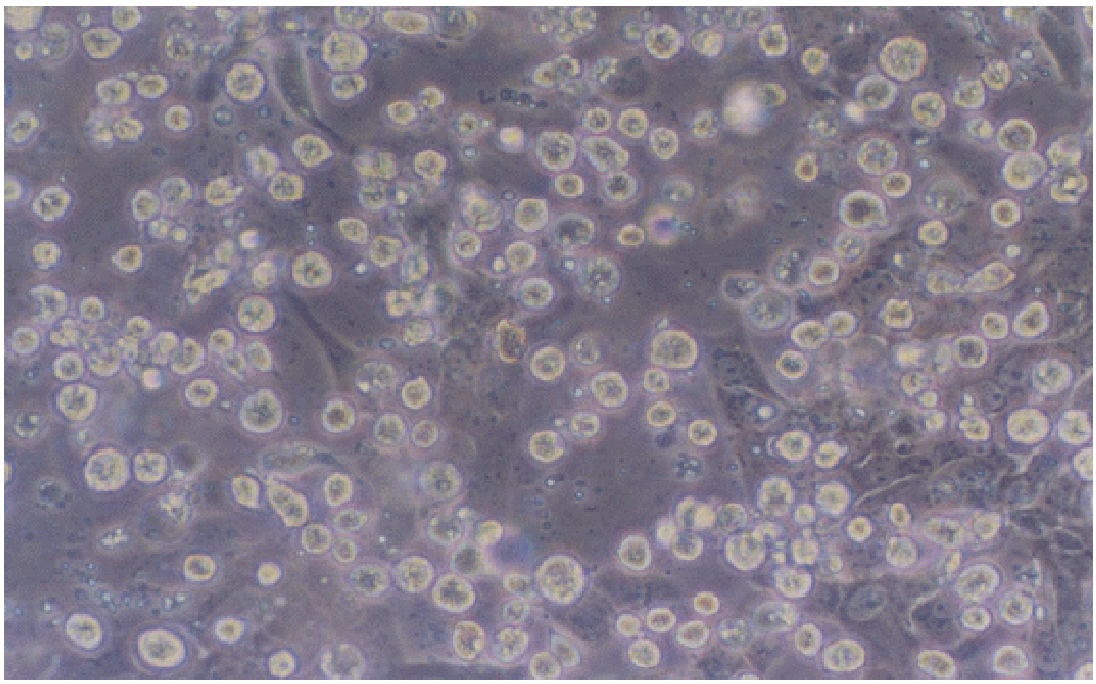

Figure 6). Influenza virus specific CPE shown on MDCK cells, (Positive, ×200)

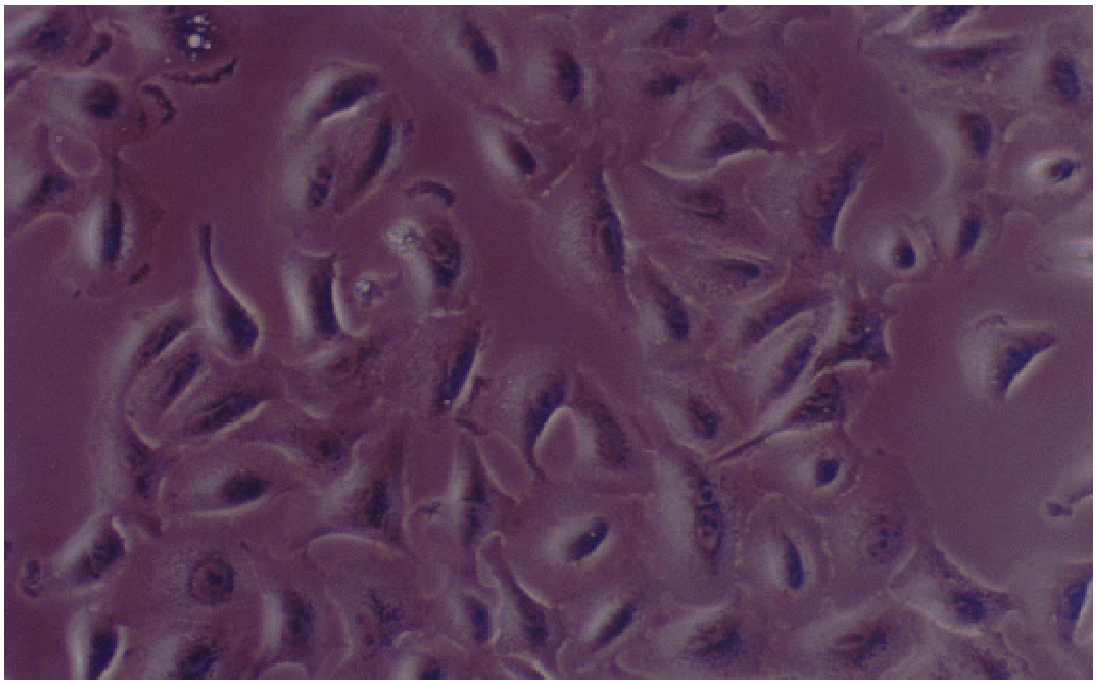

Figure 7). A549 cells inoculated with Adeno virus concentrate from the Viruskiller, ×200

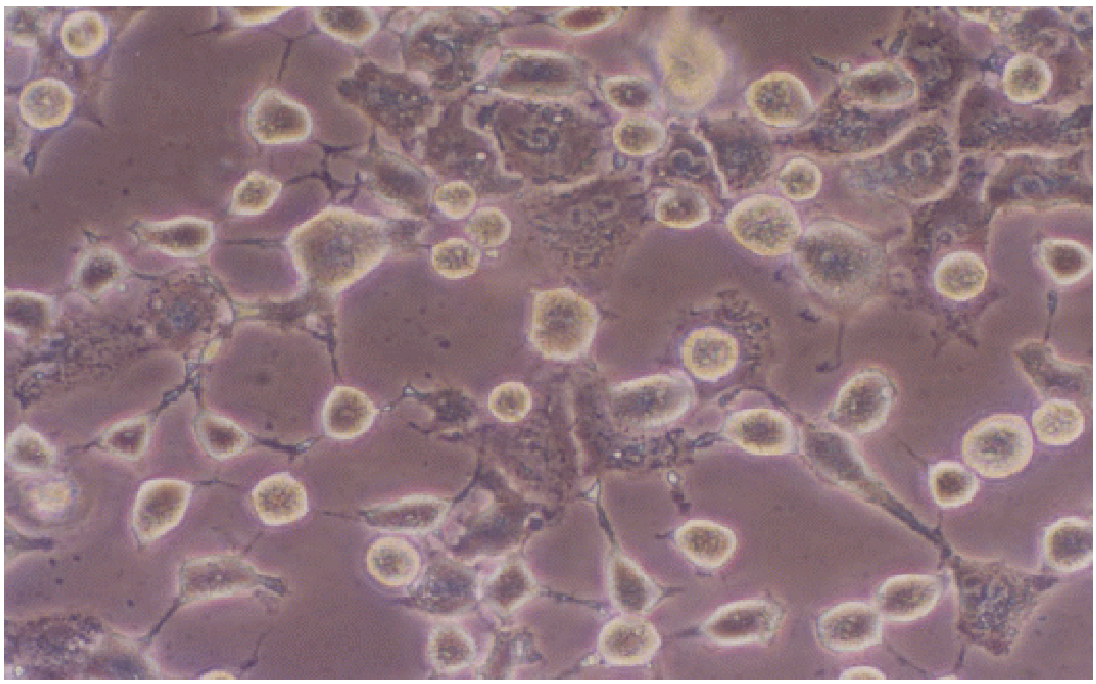

Figure 8). Adeno virus type 2 specific CPE shown on A549 cells, (Positive, ×200)

VI. Conclusion

Based on results obtained from test on inactivations of 3 selected viruses using the Viruskiller manufactured by INB Ltd, we can conclude as followings:

1) The Viruskiller could inactivate polioviruses 99.99%. Considering all the picornaviruses have the same property as poliovirus in structure and composition except antigenicity, we conclude that the Viruskiller can inactivate major common cold viruses in addition to poliovirus in air environment.

2) The Viruskiller could inactivate influenza virus 99.99% when it is used properly. Considering major respiratory viruses including mumps, measles and paramyxoviruses belong to the same myxoviruses as influenza virus, we conclude that the Viruskiller can inactivate most myxoviruses including influenza virus in air environment.

3) The Viruskiller could inactivate adenovirus 99.9%. Considering adenoviruses are large double strand DNA virus, we conclude that the Viruskiller can inactivate major large double strand DNA viruses including adenovirus, varicella and smallpox.

In summary, based on our tests on inactivation of viruses using the Viruskiller, we conclude the Viruskiller can inactivate most respiratory viruses in air environment.