TESTING THE VIRUCIDAL ACTIVITY OF APT™ T3X AGAINST INFLUENZA A VIRUS

A report prepared by Virology Research Services Ltd
for
Patient Focused Tele-Health, LLC

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Contents

Summary

Aim: To determine the antiviral activity of APT T3X against Influenza A virus.

Methods: Two methods were used to determine the antiviral activity of APT T3X on Influenza A. In the first method, the virus was incubated with APT 3TX for 15, 30, or 60 seconds, and the antiviral activity was determined by measuring the residual ability of the virus to infect cells.
In the second method, the the virus was incubated with APT 3TX for the same time points, and the virucidal activity was determined by quantifying the number of infectious virus units recovered.

Results: APT T3X treatment completely abolished IAV ability to infect a lung epithelial cell line at all the incubation times tested. Upon treatment, a decrease of 3 log of infectious particles was measured, confirming the virucidal activity of APT T3X on influenza after as little as 15 seconds.

Conclusion: Under the conditions tested, APT T3X displays 99.9% virucidal activity against Influenza A virus.

Introduction and Aims

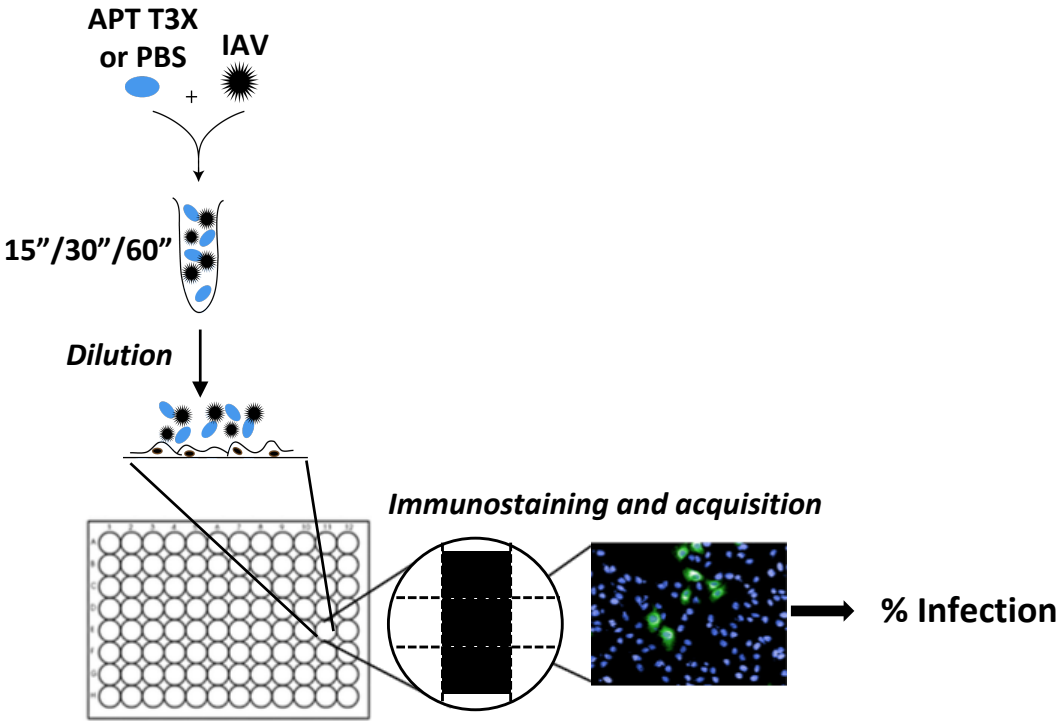
In the past 20 years the world has seen a rise in the number of viral outbreaks. In 2020, SARS-CoV2, the agent of the COVID19 pandemic, suddenly forced the world into lockdown with severe and long lasting consequences both on the economy and on global health. Therefore, it has become of paramount importance to identify new therapeutic and prophylactic treatments against infectious diseases, and especially respiratory viruses.

APT™ T3X is a proprietary topical formulation utilising the "Advanced Penetration Technology™" platform, combined with 3% Tetracycline HCl. The APT™ T3X is an FDA registered, over-the-counter, first aid antibiotic formulation in the USA.
Aim of this study was to test the antiviral activity and virucidal properties of APT™ T3X against Influenza A/WSN/33 virus.

Methods

1. Suspension test - Inhibition of infectivity

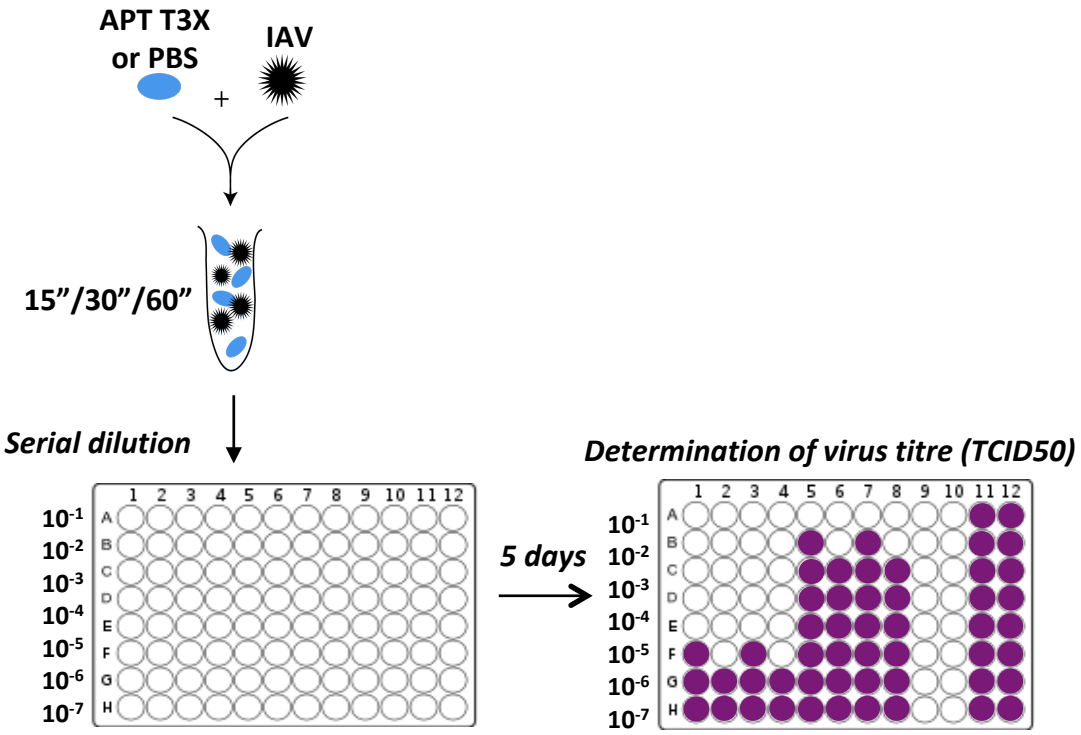
$5x10^5$ infectious units of Influenza A/WSN/33 virus (IAV) were incubated with 4 volumes of undiluted APT T3X or a PBS control.
After 15, 30, or 60 seconds, an excess of cold media was added, and $1/25^{th}$ of each reaction were added to a human lung carcinoma cell line (A549) for 1 hour.
After washing, cells were incubated for 18h at 37°C, fixed, and immuno-stained using an antibody against influenza protein NP.
The percentage of infected cells was determined by quantifying the cells expressing the NP protein. Uninfected cells were included as control.



In parallel, **a cytotoxicity test** was carried out to confirm the viability of the cells when in contact with the same concentration of ATP T3X as in the infectivity test.
The same procedure described for the infectivity assay was carried out in the absence of virus. At the end of the 18 hours incubation, a metabolic assay was carried out to determine cell viability.

2. Suspension test - Virucidal activity

To confirm that APT T3X had a direct effect on the virus, $5x10^5$ infectious units of IAV were incubated with 4 volumes of undiluted APT T3X or a PBS control.
After 15, 30, or 60 seconds, an excess of cold media was added, and the amount of infectious virus in the mixture was quantified through a serial dilution on a monolayer of MDCK-II cells.
Five days after infection, virus titre was quantified by determining the dilution at which half of the cells displayed virus-induced cytopathic effect (TCID50).



Results

1. Inhibition of infectivity

The results of the infectivity test for IAV are displayed in Figures 1 and 2. Incubation of IAV with the PBS control for 15, 30, or 60 seconds resulted in about 50% infection. Conversely, incubation of IAV with ATP-T3X for the same lengths of time completely abolished the ability of the virus to infect the cells.

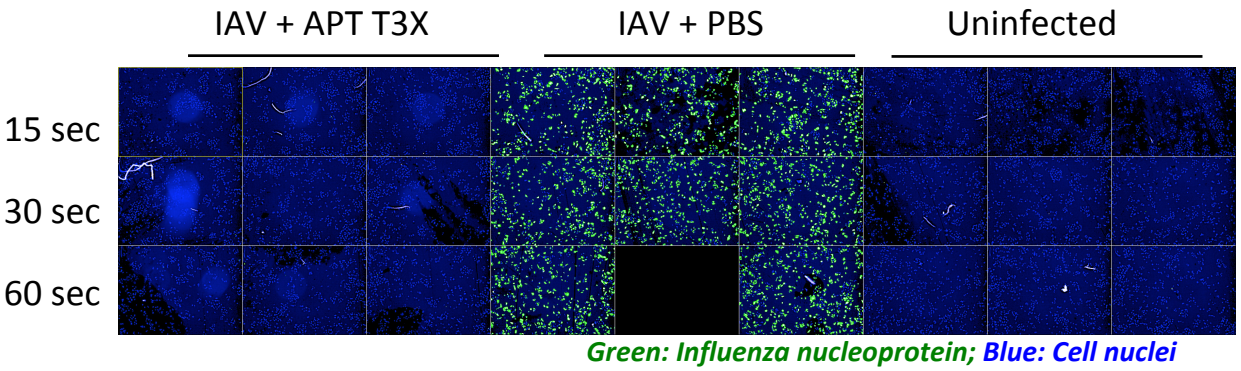
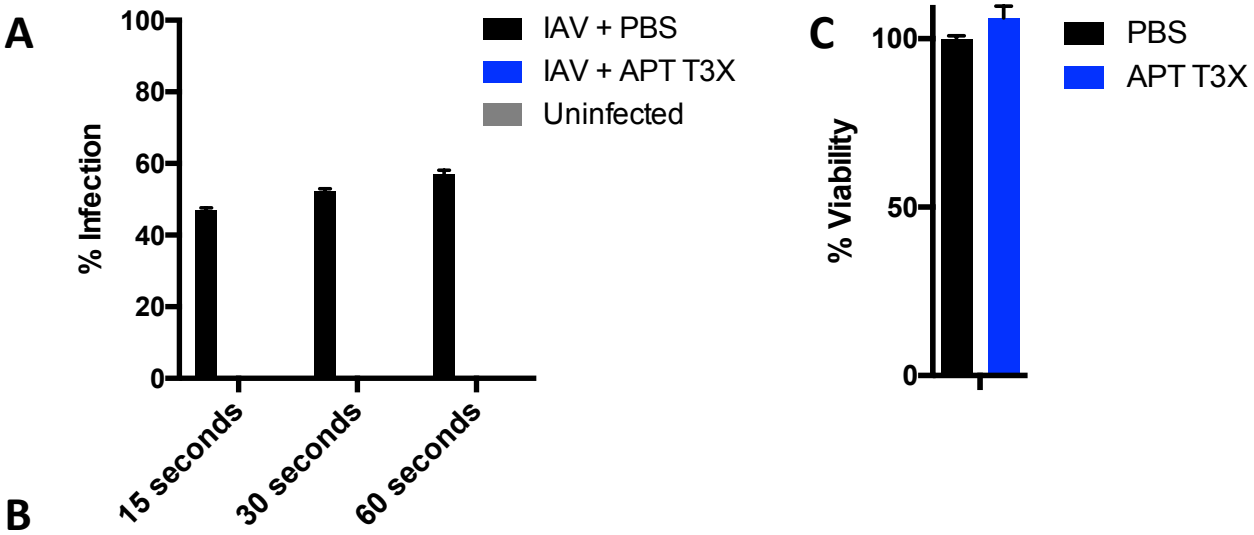


Figure 1. Representative images from the assay plate. Each square represents an area within each well for all the conditions tested. No staining is detected in the Uninfected control, while NP staining (green) is detected in approximately 50% of the cells infected with PBS-treated virus. No IAV NP staining can be detected in cells infected with APT T3X-treated IAV, suggesting that the treatment abolishes IAV infection.



B

% Infection	IAV + APT T3X			IAV + PBS			Uninfected		
15 seconds	0.11	0.07	0.13	45.65	46.86	48.20	0.10	0.11	0.14
30 seconds	0.09	0.03	0.16	52.00	50.98	53.56	0.13	0.09	0.03
60 seconds	0.10	0.06	0.09	58.32	NA	55.90	0.09	0.03	0.31

Figure 2. A. Average percentages of infection derived from the quantification of the images in Figure 1. **B.** Values plotted in A. **C.** Percentage viability of cells treated with the same concentration of APT T3X or PBS as in the infectivity assay.

Results

2. Virucidal activity

The results of the virucidal test for IAV are summarized in Figure 3. After incubation with the PBS control for 15, 30, or 60 seconds, between 3.06E+03 and 1.50E+04 TCID50/ml of IAV were recovered. Conversely, the infectious virus recovered after incubation with APT T3X for the same lengths of time was near or below the assay detection limit (1.0E+01 TCID50/ml), corresponding to a 3-log decrease in infectivity. This corresponds to a virucidal activity of 99.9%.

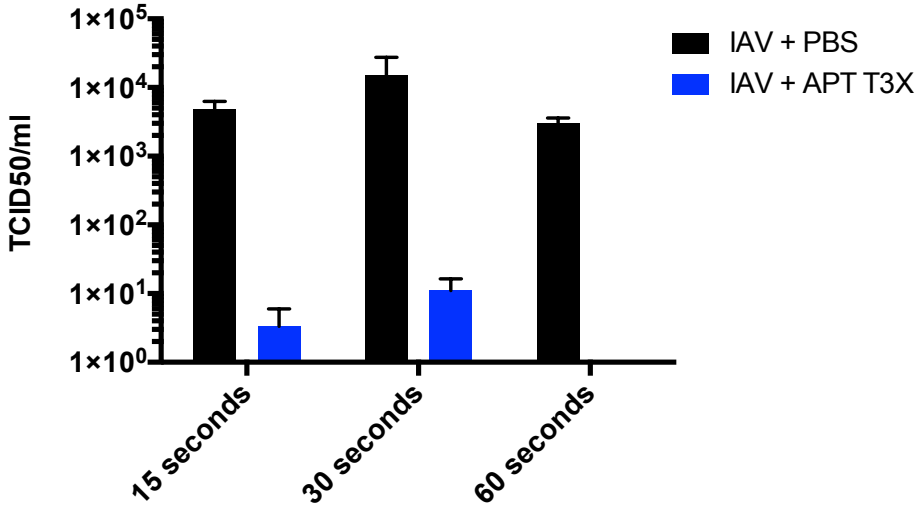


Figure 3. Averages IAV titres recovered after incubation with PBS or APT T3X for 15, 30, or 60 seconds.

Conclusions

Based on the findings reported here, exposure of Influenza A virus (H1N1; A/WSN/33) to APT T3X for 15 seconds abolished virus infectivity *in vitro* and caused a reduction of infectious virus titre of 3 logs, corresponding to a virucidal activity of 99.9%.

The result of the cytotoxicity assay confirms that the results is not a consequence of APT T3X toxicity on the readout cells, meeting the validity requirements for these tests.

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