長庚大學
Chang Gung University

Chang Gung University co-commissioned final report

Research Ttitle:
Antiviral mechanism study for 254 UVC robot system

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Executing Organization:

Emerging Viral infections Research Center, Chang Gung University

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1. BACKGROUND

Influenza A viruses are major pathogens that have considerable impact on human health during yearly epidemics. They have caused high morbidity and mortality during the pandemics that occurred in 1918, 1957, 1968, and 2009. Wild aquatic birds are the main reservoir for all subtypes of influenza A viruses and are the source for transmission to other animal species such as swines, horses and humans.

Influenza B viruses are members of the family *Orthomyxoviridae* which are known to infect only humans and seals and which were first isolated in 1940. In contrast to influenza A viruses, influenza B viruses are not divided into subtypes based on surface glycoproteins (hemagglutinin [HA] and neuraminidase [NA]) but rather are classified into two phylogenetically and antigenically distinct lineages: the B/Victoria/2/87-like (Victoria) lineage and the B/Yamagata/16/88-like (Yamagata) lineage. Both lineages currently circulate globally and evolve more slowly than human seasonal influenza A viruses, although reassortment between the Victoria and Yamagata lineages has frequently been documented.

EV71 has been implicated as the etiological agent in several large-scale outbreaks of severe neurological disorders worldwide. EV71 infections usually cause hand foot and mouth disease (HFMD) and severe neurological complications with mortality. Children are susceptible to the EV71-associated fatal pulmonary oedema and haemorrhage. In 1998, an EV71 infection epidemic occurred in Taiwan, with the virus infecting over 120000 people and killing 78 children. Many EV71 smaller scale epidemics also occurred after 1998 on the island. In recent years, many EV71 epidemics have occurred throughout the Asia-Pacific region, in Taiwan, mainland China, Malaysia, Singapore, Western Australia, the USA and Europe.

2. PURPOSE

The purpose of this study was to produce data that provides basic information about the UVC Light : 254 UVC robot system, when tested against influenza A H1N1, influenza B, and Enteorvirus 71.

3. TEST PRINCIPLE

A film of virus, dried on a glass surface, was exposed to the 254 UVC robot system for the specified exposure times at a standard distance (5 feet and 9 feet). A dried virus glass film, following exposure, the carriers were individually assayed for viral infectivity by plaque assay method.

4. VIRUS

The Influenza A/WSN/33 (H1N1), Influenza B/70555/05, Enterovirus 71/4643 virus used for this study was obtained from the Emerging Viral infections Research Center, Chang Gung University. Stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation. The supernatant was removed, aliquoted, and the high titer stock virus was stored at -70°c until the day of use. On the day of use, two aliquots of virus were removed, thawed, combined, and maintained at a refrigerated temperature until used in the assay. The stock virus tested demonstrated cytopathic effects (CPE) typical of lnfluenza virus on MDCK cells, Enterovirus 71 on RD cells.

5. TEST CELL CULTURES

The cultures of MDCK and RD cells were maintained and used at the appropriate density in tissue culture labware at 36-38°C in a humidified atmosphere of 5-7% CO_2. All cell culture documentation was retained for the cell cultures used in this assay with respert to source, passage number, growth chararteristics, seeding densities and the general condition of the cells.

6. TEST MEDIUM

The test medium used for this assay was Dulbecco's Minimum Essential Medium (DMEM) supplemented with 10% (v/v) heat inartivated fetal bovine serum. The medium was also supplemented with 10 mL gentamicin, lmL penicillin and 2.5 mL amphotericin B.

7. PREPARATION OF TEST DEVICE

The test device 254 UVC robot system were used as indicated by the Sponsor. The test device was tested while plugged in, in order to assure optimum operating conditions. The light was turned on and allowed to warm up (run) for a minimum of ten minutes immediately prior to the treatment of the carriers.

8. TEST METHOD

Preparation of Virus Films

The appropriate number of virus films were prepared by spreading a 0.1 mL aliquot of virus uniformly over a defined area, approximately 1 x 1 inches, using the lid of approximately 60 x15mm sterile glass petri dishes. For each exposure time assayed, three virus films were prepared. In addition, three films were prepared for the virus control. The virus films were air-dried 30 minutes.

Treatment of Virus Films with the UVC Light Machines

Following the completion of drying the plates (petri dish) were removed from the drying chamber and placed at least 5 feet and 9 feet from the test device set up. The device warmed up for 10 minutes and was in the "on" position. For each replicate of each exposure time, the petri dish was placed in the riser and the carrier moved so that it covered the red square on the riser. The carrier insert was gently positioned under the light machine so that the carrier lined up with the opening in the shutter mechanism and the angled edges of the riser insert were butted closely with the stationary parts of the riser. The exposure time was set on the shutter device and the expose button was pressed. The shutter opened for the exposure time and then closed on its own. The exposures were performed at ambient room conditions of 23 ℃ with a relative humidity of 33.0% inside of a biological safety cabinet.

Following the exposure time, the petri dish was removed and a 1.00 mL aliquot of test media was added to the plate. The surface of the plate was scraped with a cell scraper to resuspend the contents. Immediately, 10-fold serial dilutions of the reconstituted mixture were performed in test medium and the dilutions were assayed for infectivity plaque assay.

Treatment of Virus Control Films

Duplicate virus control films were run in parallel to the test. The virus control was held for the longest exposure time at normal room lighting inside of the biological safety cabinet. Following the exposure time, a 1 mL aliquot of test media was added to each plate and the surface of the plate was scraped with a cell scraper to resuspend the contents. Immediately, 10 fold serial dilutions of the reconstituted mixture were performed in test medium and were assayed for plaque assay.

Plaque Assay

Plaque assay are the standard method used to determine virus concentration in terms of infectious dose. Viral plaque assays determine the number of plaque forming units (pfu) in a virus sample, which is one measure of virus quantity. A confluent monolayer of host cells is infected with the virus at varying dilutions and covered with a semi-solid medium, such as agar, to prevent the virus infection from spreading indiscriminately. A viral plaque is formed when a virus infects a cell within the fixed cell monolayer. The virus infected cell will lysis and spread the infection to adjacent cells where the infection-to-lysis cycle is repeated. The infected cell area will create a plaque (an area of infection surrounded by uninfected cells) which can be seen visually or with an optical microscope. Plaque formation can take 2-3 days, depending on the virus being analyzed. Plaques are generally counted manually and the results, in combination with the dilution factor used to prepare the plate, are used to calculate the number of plaque forming units per sample unit volume (pfu/mL). The pfu/mL result represents the number of infective particles within the sample and is based on the assumption that each plaque formed is representative of one infective virus particle.

9. STUDY CONCLUSION

Taking the plaque assay results into consideration, 254 UVC robot system demonstrated a 5 feet distance, mean reduction the log reductions in viral titer were 1.699 $\log_{10}$ following a 5 min exposure time, 0.778 $\log_{10}$ following a 10 min exposure time, and 0 $\log_{10}$ following a 15 min exposure time to influenza A (H1N1), influenza B (flu B), and Enterovirus 71 (EV71) (Figure 1).

254 UVC robot system demonstrated a 9 feet distance, mean reduction the log reductions in viral titer were 2.699 $\log_{10}$ following a 5 min exposure time, 1.778 $\log_{10}$ following a 10 min exposure time, and 0 $\log_{10}$ following a 15 min exposure time to influenza A (H1N1), influenza B (flu B), and Enterovirus 71 (EV71) (Figure 2).

10. REFERENCE

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11. TABLE AND FIGURE

Figure 1. Effects of 254 UVC Disinfection Robot system Following a 5 Feet Distance, 5 MIN, 10 MIN and 15 MIN Exposure to H1N1, FLU B, and EV71 Virus

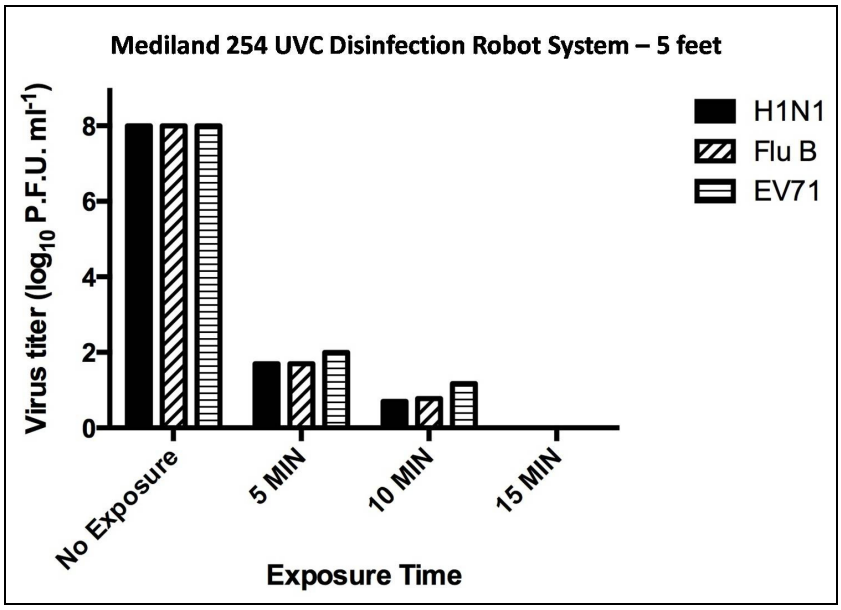
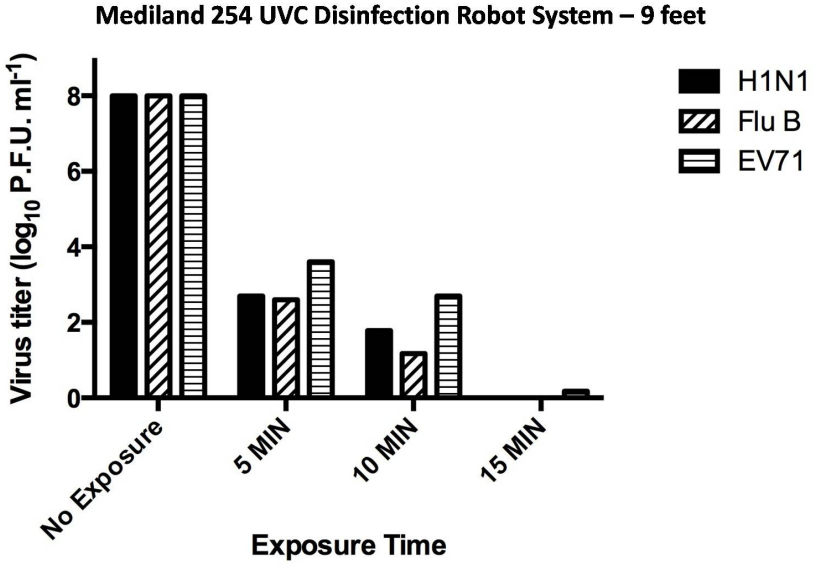


Figure 2. Effects of Mediland 254 UVC Following a 9 Feet Distance, 5 MIN, 10 MIN and 15 MIN Exposure to H1N1, FLU B, and EV71 Virus



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In the opinion of the Author, there were no circumstances that may have affected the quality or integrity of the data.

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