ประสิทธิภาพในการกำจัดไวรัสของตู้ฆ่าเชื้อด้วยแสงอัลตราไวโอเลตที่พัฒนาโดยคณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานี

Antiviral Efficiency of Ultraviolet Sterilization Chamber Developed by Faculty of Engineering, Ubon Ratchathani University

ปาริชาติ พุ่มขจร[1*] จริยาภรณ์ อุ่นวงษ์[2] ผดุง กิจแสวง[3] และ พงศ์ศักดิ์ รัตนชัยกุลโสภณ[1]

Parichat Phumkhachorn[1*] Jariyaporn Onwong[2] Padung Kitsawang[3] and Pongsak Rattanachaikunsopon[1]

[1]ภาควิชาวิทยาศาสตร์ชีวภาพ คณะวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี

[2]ภาควิชาวิศวกรรมอุตสาหการ คณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานี

[3]ภาควิชาวิศวกรรมไฟฟ้า คณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานี

[1]Department of Biological Science, Faculty of Science, Ubon Ratchathani University

[2]Department of Industrial Engineering, Faculty of Engineering, Ubon Ratchathani University

[3]Department of Electrical Engineering, Faculty of Engineering, Ubon Ratchathani University

*E-mail: scpariph@gmail.com

Received: Oct 26, 2022

Revised: Dec 02, 2022

Accepted: Dec 06, 2022

บทคัดย่อ

ในสถานการณ์โรคระบาด อุปกรณ์ทางการแพทย์มักมีราคาที่สูงขึ้นมาก ด้วยเหตุนี้ทางคณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานีจึงได้สร้างตู้ฆ่าเชื้อด้วยแสงอัลตราไวโอเลต (ตู้ฆ่าเชื้อด้วยแสงยูวี) ขึ้น เพื่อให้มีอุปกรณ์ทางการแพทย์ที่มีราคาไม่สูงไว้ใช้ในสถานการณ์ดังกล่าว การศึกษานี้มีวัตถุประสงค์เพื่อทดสอบความสามารถของตู้ฆ่าเชื้อด้วยแสงยูวีที่พัฒนาขึ้นโดยคณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานี ในการยับยั้งเชื้อไวรัส โดยการศึกษานี้ใช้ coliphage ซึ่งเป็นไวรัสที่สามารถบุกรุก *Escherichia coli* เป็นตัวแทนของไวรัส การศึกษาผลของเวลาที่ใช้ในการอบแสงยูวีต่อความสามารถของตู้ฆ่าเชื้อด้วยแสงยูวีในการยับยั้ง coilphage ทำโดยนำ coliphage ที่มีความเข้มข้น 10^5 pfu/mL ปริมาตร 10 ไมโครลิตรหยดลงบนจานอาหารเลี้ยงเชื้อ BHI agar แล้วนำไปบ่มในตู้ฆ่าเชื้อด้วยแสงยูวีเป็นเวลา 120, 60, 30, 20 และ 10 วินาที จากนั้นเททับ BHI agar ที่มีหยดของ coliphage อยู่ ด้วย soft BHI agar ที่มี *E. coli* ผสมอยู่ แล้วนำไปบ่มที่อุณหภูมิ 37 องศาเซลเซียส เป็นเวลา 24 ชั่วโมง พบว่าการอบแสงยูวีเป็นเวลาตั้งแต่ 30 วินาทีสามารถยับยั้ง coliphage ได้อย่างสมบูรณ์ การศึกษาผลของปริมาณไวรัสต่อความสามารถของตู้ฆ่าเชื้อด้วยแสงยูวีในการยับยั้ง coilphage ทำโดยนำ coliphage ที่มีความเข้มข้น 10^6, 10^7 และ 10^8 pfu/mL ปริมาตร 10 ไมโครลิตรหยดลงบนจานอาหารเลี้ยงเชื้อ BHI agar แล้วนำไปบ่มในตู้ฆ่าเชื้อด้วยแสงยูวีเป็นเวลา 30 วินาที จากนั้นเททับ BHI agar ที่มีหยดของ coliphage อยู่ ด้วย soft BHI agar ที่มี *E. coli* ผสมอยู่ แล้วนำไปบ่มที่อุณหภูมิ 37 องศาเซลเซียส เป็นเวลา 24 ชั่วโมง พบว่าการอบแสงยูวีเป็นเวลา 30 วินาที สามารถยับยั้ง coliphage ทุกความเข้มข้นที่ใช้ในการศึกษานี้ได้อย่างสมบูรณ์ จากการศึกษานี้ทำให้สามารถสรุปได้ว่าหากต้องนำตู้ฆ่าเชื้อด้วยแสงยูวีนี้ไปใช้งานจริงและต้องการให้ยับยั้งไวรัสได้อย่างสมบูรณ์ควรใช้เวลาในการอบแสงยูวีเป็นเวลาอย่างน้อย 30 วินาที

คำสำคัญ: ประสิทธิภาพในการกำจัดไวรัส ตู้ฆ่าเชื้อด้วยแสงอัลตราไวโอเลต ไวรัส

Abstract

In pandemic situations, prices of medical equipment tend to increase substantially. Therefore, the faculty of Engineering, Ubon Ratchathani University has developed an ultraviolet sterilization chamber (UV chamber) in order to provide affordable medical equipment in such situations. This study aimed to examine the ability of the UV chamber developed by the Faculty of Engineering, Ubon Ratchathani University to inhibit a virus. In this study, coliphage, a virus infecting *Escherichia coli*, was used as a representative of viruses. To examine the effect of exposure time to UV on inhibitory ability of the UV chamber against coliphage, 10 mL of coliphage with the concentration of 10^5 pfu/mL were spotted onto BHI agar and then subjected to UV exposure for 120, 60, 30, 20 and 10 sec. After UV exposure, BHI agar having UV exposed coliphage was overlaid with soft BHI agar containing *E. coli* and then incubated at 37°C for 24 h. It was found that UV treatment for 30 sec or more could completely inhibit coliphage. To examine the effect of viral load on inhibitory ability of the UV chamber against coliphage, 10 mL of coliphage with the concentrations of 10^6, 10^7 and 10^8 pfu/mL were spotted onto BHI agar and then subjected to UV exposure for 30 sec. After UV exposure, BHI agar having UV exposed coliphage was overlaid with soft BHI agar containing *E. coli* and then incubated at 37°C for 24 h. It was found that UV treatment for 30 sec could completely inhibit all concentrations of coliphage used in this study. From this study, it is suggested that the most effective exposure time for the application of the UV chamber to completely inhibit viruses would be at least 30 sec.

Keywords: Antiviral efficiency, Ultraviolet sterilization chamber, Virus

1. Introduction

Ultraviolet (UV) is radiation or light that is naturally emitted from the sun to the earth. It has wavelengths in the range of 100 to 400 nm and can be categorized into three types based on wavelength including UVA, UVB and UVC [1]. The sun's UV rays that reach the earth are composed primarily of UVA (90%-95%) and UVB (5%-10%), with UVC absorbed by the ozone layer [2]. UVA is a type of ultraviolet radiation with the longest wavelengths (315 to 400 nm). Due to the longer wavelengths, it can penetrate deeper into the skin and reach the middle layer of the skin called the dermis causing premature aging, indirect DNA damage and oxidative stress [2]. UVB has shorter wavelengths (280-315 nm) than UVA. It has the potential to reach the outer layer of the skin which is called the epidermis. It has the potential to damage only the outer layers of the skin (epidermis) causing sunburn, skin inflammation, DNA damage, and eye damage [2]. UVC with wavelengths between 100 and 280 nm is UV light that cannot penetrate the earth's atmosphere. Because UVC is high energy compared to the other two types of UV, it is capable of killing various microorganisms. Therefore UVC is often referred to as germicidal UV [3]. As mentioned above, UVC from the sun is not readily available for use. Thus, UVC used to kill microorganisms has to be produced from artificial radiation sources. At present, UVC lamps are produced for commercial sale for disinfection use in places such as hospital wards, aseptic laboratory and swimming pool.

The mechanism by which UVC kills microorganisms is by impairing the structure of nucleic acids, an important component of DNA. The most common effect of UVC on DNA is the pyrimidine dimers [4], caused by covalent linkages between two consecutive pyrimidine bases along the nucleotide chain. This DNA damage gives the DNA a distorted structure and is unable to replicate itself [5].

In pandemic situations, the need of materials and equipment for disease prevention or sterilization is much higher than the production capacity resulting in shortage or increase in the price of such

equipment. For this reason, the Faculty of Engineering, Ubon Ratchathani University, Thailand intends to develop an ultraviolet sterilization chamber (hereafter as UV chamber) for disinfection of viruses. The device consists of two 8-watt UV lamps. Although there have been research reports showing that UV light has the ability to kill many types of viruses including bacteriophages [6], influenza virus [7], adenovirus [8], poliovirus [8], Ebola [9], MERS-CoV [9] and SARS-CoV-2 [10], in order to put the UV chamber into practice, it is necessary to test its effectiveness in inhibiting viruses. Therefore, the objective of this study was to test the ability of the UV chamber to inactivate a virus and to examine the effects of UV exposure time and viral load on the efficiency of the UV chamber in inactivating a virus. In this study, coliphage, a bacteriophage, was used as a representative of viruses for biosafety reasons. It is a bacteriophage that can invade and destroy *Escherichia coli*. Both coliphage and *E. coli* are safe microorganisms and do not cause disease in human.

2. Materials and methods

2.1. UV chamber

The UV chamber used in this study was 44 cm wide, 40.5 cm long and 44 cm high with a front swing-hinged door. Two 8-watt UV lamps (TUV TL Mini) were placed at the ceiling inside the chamber (Figure 1). A time setting knob placed at the upper right corner outside the chamber facilitated time setting in minute from 1-30 minutes.

2.2. Microorganisms

The bacteriophage (or phage) used in this study was coliphage, a bacteriophage isolated from water collected from a sewerage pond. The coliphage host bacterium was *Escherichia coli* ATCC 25922, a bacterium belonging to biosafety level 1.

2.3. Infectivity of coliphage against *E. coli*

To test the ability of coliphage to inhibit *E. coli*, 10 µL of approximately 10^5 pfu/mL of coliphage were dropped onto the center of a BHI agar plate. Four mL of soft agar (0.4% agar) containing 100 µL of *E. coli* were poured over the agar plate with a drop of coliphage. The plate was incubated at 37°C for 24 h to observe an inhibition zone where *E. coli* was inhibited by coliphage. For the control, BHI broth was used instead of coliphage to inoculate the BHI agar plate. This experiment was performed in triplicate.

2.4. Antiviral activity of UV chamber

To test the ability of the UV chamber to inactivate coliphage, 10 µL of approximately 10^5 pfu/mL of coliphage were dropped onto the center of a BHI agar plate. The plate was exposed by UV in the UV chamber in such a way that the top cover of the agar plate was opened. After UV exposure for 2 min, 4 mL of soft agar (0.4% agar) containing 100 µL of *E. coli* were poured over the UV treated plate with a drop of coliphage. The plate was incubated at 37°C for 24 h to observe an inhibition zone where *E. coli* was inhibited by coliphage. The control was prepared as mentioned above except no UV irradiation to ensure the normal lytic activity of coliphage. This experiment was performed in triplicate.

2.5. Effect of UV exposure time on antiviral activity of UV chamber

This experiment was designed to find the optimal UV exposure time to inactivate coliphage used in this study. The experiment was performed as described above for testing antiviral activity of the UV chamber with the UV exposure times of 10, 20, 30, 60 and 120 sec. This experiment was performed in triplicate for each UV exposure time.

2.6. Effect of viral concentration on antiviral activity of UV chamber

This experiment was designed to examine the effect of coliphage concentration on antiviral activity of the UV chamber. The experiment was performed as described above for testing antiviral activity of the UV chamber with coliphage concentrations of 10^6, $10^7$and 10^8 pfu/mL and the UV exposure time of 30 sec.

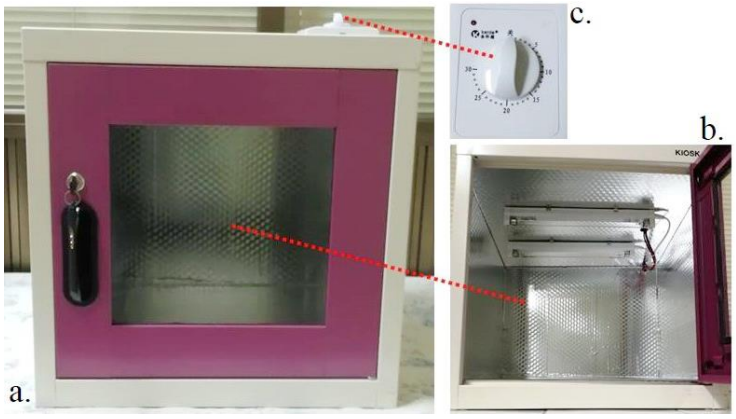

Figure 1 Composition of ultraviolet sterilization chamber (UV chamber)
(a.) front view of UV chamber; (b.) inside view of UV chamber; (c.) time setting knob

3. Results and discussion

3.1. Infectivity of coliphage against *E. coli*

When 10 µL of coliphage with a concentration of 10^5 pfu/mL were dropped onto a BHI agar plate, overlaid with soft BHI agar containing *E. coli* and incubated at 37°C for 24 h, an inhibition zone was found on the plate where coliphage was dropped (Figure 2a). On the other hand, no inhibition zone was found on the control plate where BHI broth (instead of coliphage) was dropped (Figure 2b). This experiment confirmed that coliphage used in this study was active against *E. coli*.

Testing the ability of coliphage to inhibit *E. coli* is an important step and must be done first in this study. This is because it can confirm that coliphage used in this study still have lytic activity against *E. coli*, indicated by an inhibition zone where *E. coli* cells are killed by coliphage and cannot grow. Storing phages at 4°C has been considered to be the most preferred method of preserving phages, as it is convenient and economical. It was reported that storing phages with this method for a long time (more than 1 year) did not cause phages to lose their lytic ability against their specific host cells [11]. However, there were some reports showing that storing phages with this method was ineffective and tended to decrease lytic activity of phages [12]. Therefore, it is of importance to determine lytic activity of coliphage used in this study which has been stored at 4°C for some time (more than one year) before using it in further experiments.

3.2. Antiviral activity of UV chamber

Coliphage with a concentration of 10^5 pfu/mL inoculated onto a BHI agar plate was exposed to UV for 2 min in the UV chamber and then poured over with soft BHI agar containing *E. coli*. After incubation at 37°C for 24 h, no inhibition zone was observed (Figure 3a) indicating the complete inactivation of coliphage by UV. However, coliphage unexposed to

UV on the control plate still had lytic activity against *E. coli* causing an inhibition zone on the plate (Figure 3b). The results demonstrated that exposure with UV for 2 min in the UV chamber was able to inactivate coliphage.

This experiment was a preliminary test to determine whether the UV chamber was capable of inactivating coliphage by exposing coliphage to UV for 2 min. Since the UV intensity at the place where the agar plate was placed in the UV chamber was approximately 1.5 mW/cm^2, exposure to UV for 2 min (or 120 sec) made that place having the UV dose of 180 mJ/cm^2 (determined by multiplying the UV intensity by the exposure time in seconds). This number was in the range of UV doses previously reported to kill bacteria and viruses which were between 1 and 400 mJ/cm^2 depending on the types of microorganisms [13]. From the experiment, it was found that UV exposure at the dose of 180 mJ/cm^2 (or for 2 min) was able to completely inactivate coliphage. This was evident by the normal growth of *E. coli* (no inhibition zone) at the site where coliphage was dropped onto the agar plate. UV light has been reported to kill microorganisms by causing abnormalities in their DNA, especially pyrimidine dimers [4]. They could deter DNA to undergo DNA replication and transcription [5], leading to eventual microbial death. Different microorganisms are resistant to UV light differently depending on their structures and ability to repair DNA damaged by UV light [14].

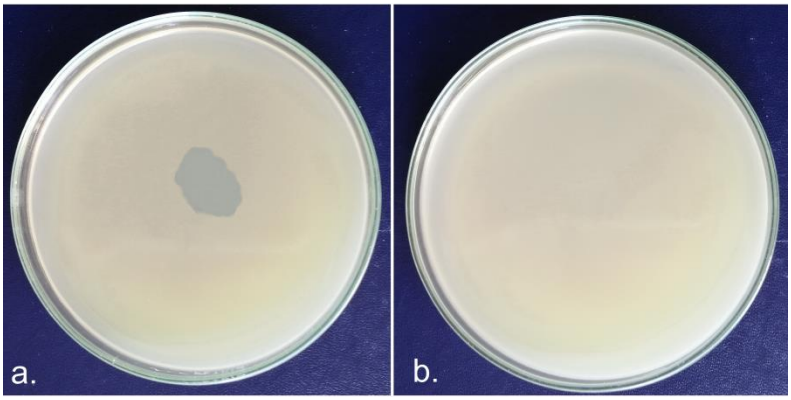

Figure 2 Lytic activity of coliphage against *E. coli*
(a.) The treatment set with coliphage; (b.) The control set without coliphage

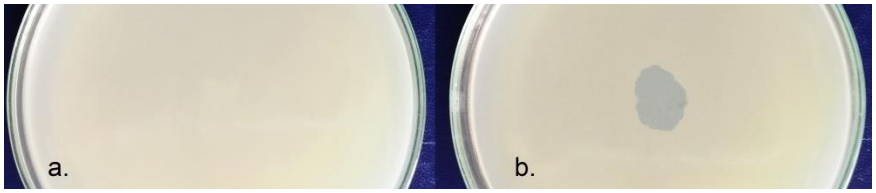

Figure 3 Antiviral activity of UV exposure for 2 min against coliphage (10^5 pfu/mL)
(a.) The treatment set with UV exposure; (b.) The control set without UV exposure

3.3. Effect of UV exposure time on antiviral activity of UV chamber

Coliphage with a concentration of 10^5 pfu/mL inoculated onto a BHI agar plate was exposed to UV for 10, 20, 30, 60 and 120 sec in the UV chamber and then poured over with soft BHI agar containing *E. coli*. After incubation at 37°C for 24 h, no inhibition zones were observed in the treatment sets exposed to UV for 30, 60 and 120 sec (Table 1, Figure 4) indicating the complete inactivation of coliphage by UV. On the other hand, inhibition zones were observed in the treatment sets exposed to UV for 10 and 20 sec (Table 1, Figure 4) with the sizes of inhibition zone inversely proportional to exposure times. For the control without UV exposure, coliphage still had lytic activity against *E. coli* causing an inhibition zone on the plate (Figure 4). The results demonstrated that the optimal UV exposure time of the UV chamber to completely inactivate coliphage was 30 sec.

This experiment was designed to find the optimal UV exposure time for the UV chamber to completely inactivate coliphage by reducing UV exposure time from 2 min (120 sec) to 60, 30, 20, and 10 sec. By reducing exposure time, UV dose was also reduced. For exposure times of 60, 30, 20, and 10 sec, UV doses were 90, 45, 30 and 15 mJ/cm^2, respectively. This experiment found that exposure to UV for 30 and 60 sec completely inactivated coliphage. This result was consistent with previous reports showing that UV doses in the range of 1 to 400 mJ/cm^2 could kill bacteria and viruses [13]. At UV exposure time of 20 sec, small clear plaques were found throughout the site where coliphage was applied onto the agar plate. At this UV exposure time, most of the coliphage was inactivated by UV, leaving few particles of coliphage to lyse *E. coli* cells, resulting in clear spots (plaques) instead of an inhibition zone.

3.4. Effect of viral concentration on antiviral activity of UV chamber

Coliphage with concentrations of 10^6, $10^7$and $1\,0^{\,8}$ pfu/mL were inoculated onto BHI agar plates exposed to UV for 30 sec, the optimal time for coliphage inactivation. After overlaid with soft BHI agar containing *E. coli* and incubated at 37°C for 24 h, no inhibition zone was observed for all treatments set with different concentrations of coliphage (Table 2) indicating the complete inactivation of coliphage by UV. Normal lytic activity of coliphage against *E. coli*, indicated by an inhibition zone, was still observed in the control. The results showed that UV exposure of coliphage for 30 sec in the UV chamber completely inhibited coliphage with all concentrations used in this experiment.

This experiment investigated the effect of coliphage concentrations on the UV chamber's ability to inactivate coliphage using the minimum time (30 sec) required to completely kill coliphage at a concentration of 10^5 pfu/mL. Coliphage concentrations used in this experiment were tested at 10^6, 10^7 and 10^8 pfu/mL which were 10, 100 and 1000 times higher than that used in previous experiments (10^5 pfu/mL), respectively. These concentrations were considered to be higher than those found contaminated in the environments [15]. This is because viruses (including phages) are normally present in small quantities in the environment and unable to multiply on their own [16]. Upon entering their specific host cells, they multiply to increase their number. In this experiment, it was found that UV exposure for 30 sec was able to inactivate coliphage at all concentrations used. The reason for this may be that coliphage is too small to block each other from UV exposure even when it is highly concentrated.

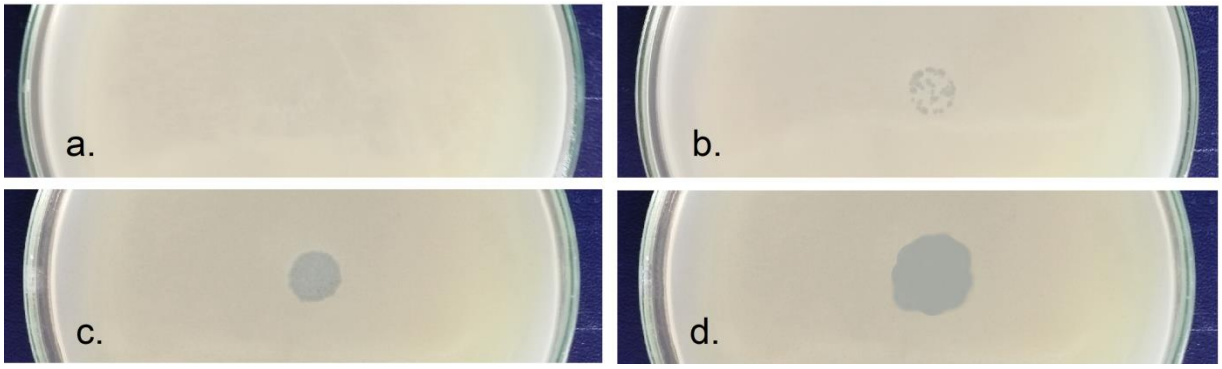

Figure 4 Antiviral activity of the UV chamber against coliphage (10^5 pfu/mL) at different UV exposure times (a.) 30 sec; (b.) 20 sec; (c.) 10 sec; (d.) control (no UV exposure)

Table 1 Antiviral activity of the UV chamber against coliphage (10^5 pfu/mL) at different UV exposure times

UV exposure time (sec)	Antiviral activity
0 (control)	-
10	-
20	-
30	+
60	+
120	+

- = absence of antiviral activity against coliphage; + = presence of antiviral activity against coliphage

Table 2 Antiviral activity of the UV chamber against coliphage with different concentrations when exposed to UV for 30 sec

Concentration of coliphage (pfu/mL)	Antiviral activity
10^6	+
10^7	+
10^8	+

- = absence of antiviral activity against coliphage; + = presence of antiviral activity against coliphage

4. Conclusion

Antiviral ability of the UV chamber developed by the faculty of Engineering, Ubon Ratchathani University was examined by using coliphage, a bacteriophage specific to *E. coli*, as a representative of viruses for biosafety reasons. The UV chamber was shown to have antiviral ability against coliphage and the optimal UV exposure time was 30 sec. By using this UV exposure time, coliphage with all concentrations used in this study was completely inactivated. This study suggested that the UV chamber might be useful for inactivating viruses with an awareness of the dependence of UV exposure time on types of viruses.

5. References

[1] Dale Wilson, B., Moon, S. and Armstrong, F. 2012. Comprehensive review of ultraviolet radiation and the current status on sunscreens. **The Journal of Clinical and Aesthetic Dermatology.** 5(9): 18-23.

[2] Amaro-Ortiz, A., Yan, B. and D'Orazio, J.A. 2014. Ultraviolet radiation, aging and the skin: Prevention of damage by topical cAMP manipulation. **Molecules.** 19(5): 6202-6219.

[3] Chiappa, F. and et al. 2021. The efficacy of ultraviolet light-emitting technology against coronaviruses: A systematic review. **Journal of Hospital Infection.** 114: 63-78.

[4] Douki, T., von Koschembahr, A. and Cadet, J. 2017. Insight in DNA repair of UV-induced pyrimidine dimers by chromatographic methods. **Photochemistry and Photobiology.** 93(1): 207-215.

[5] Banas, A.K. and et al. 2020. All you need is light. photorepair of UV-induced pyrimidine dimers. **Genes (Basel).** 11(11): 1304.

[6] Clark, E.M. and et al. 2012. Inactivation of recombinant bacteriophage lambda by use of chemical agents and UV radiation. **Applied and Environmental Microbiology.** 78(8): 3033-3036.

[7] McDevitt, J.J., Rudnick, S.N. and Radonovich, L.J. 2012. Aerosol susceptibility of influenza virus to UV-C light. **Applied and Environmental Microbiology.** 78(6): 1666-1669.

[8] Lamont, Y. and et al. 2007. Pulsed UV-light inactivation of poliovirus and adenovirus. **Letters in Applied Microbiology.** 45(5): 564-567.

[9] Eickmann, M. and et al. 2018. Inactivation of Ebola virus and Middle East respiratory syndrome coronavirus in platelet concentrates and plasma by ultraviolet C light and methylene blue plus visible light, respectively. **Transfusion.** 58(9): 2202-2207.

[10] Biasin, M. and et al. 2021. UV-C irradiation is highly effective in inactivating SARS-CoV-2 replication. **Scientific Reports.** 11: 6260.

[11] Alvi, I.A. and et al. 2018. Storage of bacteriophages at 4°C leads to no loss in their titer after one year. **Pakistan Journal of Zoology.** 50(6): 2395-2398.

[12] Golec, P. and et al. 2011. A reliable method for storage of tailed phages. **Journal of Microbiological Methods.** 84(3): 486-489.

[13] Dai, T. and et al. 2012. Ultraviolet C irradiation: an alternative antimicrobial approach to localized infections? **Expert Review of Anti-Infective Therapy.** 10(2): 185-195.

[14] Pereira, R.V. and et al. 2014. Evaluation of the effects of ultraviolet light on bacterial contaminants inoculated into whole milk and colostrum, and on colostrum immunoglobulin G. **Journal of Dairy Science.** 97(5): 2866-2875.

[15] Prussin, A.J., Garcia, E.B. and Marr, L.C. 2015. Total virus and bacteria concentrations in indoor and outdoor air. **Environmental Science & Technology Letters.** 2(4): 84-88.

[16] Phumkhachorn, P. and Rattanachaikunsopon, P. 2019. Bacteriophages: Biology and applications. **Journal of Science and Technology, Ubon Ratchathani University.** 21(3): 1-13. (*in Thai*)