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# Effect of Ultraviolet C (UVC) Light for Room Disinfection

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## Abstract

The effect of manual cleaning and disinfection depend on how careful it is done, and the technique used. Disinfection with $H_2O_2$, acidic acid or ozon products are all based on chemicals with toxic effects. Ultraviolet light C is a chemical free disinfection method which do not demand sealing of the room before the disinfection and ventilation after disinfection. The aim of this study was to test the effect of a new disinfection system for room disinfection using UVC light.

For laboratory test eight different strains of well-known clinical isolates of test bacteria were used and placed on a laminated wood plate and exposed to UVC light up to 90 minutes. For field test, imprints were taken from five spots before and after exposure to UVC light in an outpatient clinic.

Gram negative bacteria were reduced about 8 $\log_{10}$ at 3m and 6 $\log_{10}$ at 5m. Gram positive bacteria were less susceptible to UVC light the reduction was around 6 $\log_{10}$ at 3m and around 5 $\log_{10}$ at 5m. Vancomycin resistant *Enterococcus faecium* seem to be less sensitive to UVC than other Gram-positive bacteria. The bacteria distribution on each spot in the field test were the same before and after UVC but the number of bacteria was clearly reduced during the disinfection. Chairs were the most contaminated spots.

In conclusion, UVC light is an effective method, to kill bacteria in the laboratory, but also very effective to disinfect patients' rooms in short time.

***Keywords:*** *Ultraviolet Light; UVC; Room Disinfection; C. difficile; VRE; A. baumannii; S. aureus; Laboratory Test; Field Test*

## Introduction

Cleaning and disinfection of patient's rooms in hospitals is a great challenge as many nosocomial infections are expected to be caused by microorganisms from the environment [1,2]. The effect of manual cleaning and disinfection depend on how careful it is done, and the technique used as microorganisms may be spread by the cleaning procedure [3,4]. In the last decades several different systems for room disinfection have been developed to ensure disinfection of areas that may be difficult to clean manually.

Chemical methods for room disinfection have used aerosolized or vapored hydrogen peroxide with or without silver ions [5,6]. Solid peracetic acid, vapored or aerosolized peracetic acid alone or in combinations with hydrogen peroxide and acetic acid have been used

[5-7]. Vapored or aerosolized ozon in various concentrations have also been developed [8,9]. Disinfection with $H_2O_2$, acidic acid or ozon products are all based on chemicals with toxic effects and skin irritation. Thus, the room must be sealed carefully before disinfection and it need time for thorough ventilation after the disinfection, often for several hours [6]. In many hospital settings it is crucial to minimize the turnover time for the room before it is to be occupied by a new patient. Time for effective cleaning and disinfection is therefore an important parameter for effective use of hospital beds.

Ultraviolet light C is a chemical free disinfection method which do not demand sealing of the room before the disinfection and the room do not need ventilation after disinfection. Thus, UVC light only need the time that are needed for the disinfection [6].

Many of the room UVC disinfection instruments are manufactured by several companies and the recommendations for use may differ from company to company even when based on the same principles. This depend on technical specifications of the instruments, time of disinfection, number of disinfection cycles recommended. The time for disinfection is in advance of UV-light disinfection as well as it is free of toxic chemicals.

Both the chemical methods and UVC seem to be effective against many virus [10-13]. The effect on protozoa is very limited [14,15]. The methods are effective on most vegetative bacteria whereas the active effect upon bacteria spores is more doubtful [1,2,6].

## Aim of the Study

The aim of this study was to test the effect of a new disinfection system for room disinfection using UVC light (Dolphin Care UVC unit) on vegetative bacteria and preparations of pure bacteria spores at different distances and different exposure times.

## Materials and Methods

The disinfection unit consist of a central unit with 8 UVC lamps and two satellite units with two UVC lamps each (Figure 1). The satellites can be placed in small rooms such as toilets, under beds or at other dead spaces.

***Figure 1:*** *Dolphin Care UVC disinfection unit.*

The measured doses (J/m$^2$) of exposure of UVC at different distances and levels are shown in table 1.

| Test of room disinfection unit at Rigshopitalet, Denmark Dose (J/m$^2$) after 15 minutes | | | | | |
|---|---|---|---|---|---|
| Height | Distance in meter from room disinfection unit | | | | |
| | 1 | 2 | 3 | 4 | 5 |
| 222 cm | 3.805 | 3.006 | 2.020 | 893 | 705 |
| 148 cm | 13.906 | 5.555 | 2.580 | 1.684 | 728 |
| 112 cm | 18.269 | 6.009 | 2.755 | 1.431 | 746 |
| 74 cm | 20.017 | 6.484 | 2.934 | 1.816 | 770 |
| 43 cm | 16.295 | 5.718 | 3.044 | 1.847 | 779 |
| At floor | 11.793 | 5.505 | 3.047 | 1.804 | 820 |

***Table 1***

### Test design for laboratory test on bacteria

For laboratory test eight different strains of well-known clinical isolates of test bacteria were used in five tenfold dilutions. 250 µl of each dilution was placed on a square of 5 x 5 cm. on a laminated wood plate. Identical wood plates were places up to 5 meters from the UVC unit. The UVC light was turned on up to 90 min. The reduction of bacteria caused by UVC light was measured by the reduction in vital bacteria after UVC exposure compared to the initial number of bacteria.

### Test design for field test

For field test, imprints were taken from five spots before exposure to UVC light in an outpatient clinic and from the same spots after exposure to UVC light for up to 60 minutes.

### Laboratory test

### Test bacteria

- *Acinetobacter baumannii*, strain no. 3262/10H
- *Stenotrophomonas maltophilia* strain no. 2140/17H
- *Staphylococcus aureus*, strain no. 2622/12H
- *Klebsiella pneumoniae*, strain no. 2284/12H
- *Enterococcus faecalis*, strain no. 3758/12H
- Vancomycin resistant *Enterococcus faecium* (VRE) strain no. 296411/17U
- *Clostridium difficile* (spores), strain no. 47832
- *Bacillus cereus* (spores), strain no. 31553/11A.

Clinical isolates of three relative insusceptible disinfectants Gram negative rods (*A. baumannii, S. maltophilia* and *K. pneumoniae*), three Gram positive cocci (*S. aureus*, vancomycin resistant *E. faecium* and *E. faecalis*) and spores from two spore forming Gram positive rod (*C. difficile* and *B. cereus*) were used as representative bacteria for the laboratory experiments.

### Dilutions of bacteria

10-fold dilutions from about $10^8$ CFU/ml to about $10^3$ of *A. baumannii, S. maltophilia, B. cereus, S. aureus*, *K. pneumoniae* and *E. faecalis* from a 24-hour culture were made.

10-fold dilutions from about $10^6$ CFU/ml to about $10^3$ of *C. difficile* or *B. cereus* from a 24-hour culture: was made.

The dilutions were selected based on the experience from previous pilot studies.

### Control culture of bacteria dilutions

The three lowest concentrations of all bacteria were control cultured and CFU counted.

### Test plates

Four laminated wood plates with 24 squares of 5 x 5 cm. on each plate were used for the laboratory test.

### Inoculation of test plates

250 µl of each bacteria suspension was added and spread equally to a square of 5 x 5 cm corresponding to 10 µl pr. $cm^2$. Thus, the number of bacteria ranged from 10 to 1,000,000 bacteria pr. $cm^2$.

### Placing the plates

The plates were placed 1, 2, 3, 4 and 5m from the UVC unit approximately 75 cm. above the floor. One plate was kept unexposed to UVC in a separate room and served as control for spontaneous death of the bacteria.

### Exposure to UVC light

High UVC exposure was used in the experiments (see technical data). In separate experiments the exposure time was 90 min, 60 min, 45 min, 30 min and 15 minutes.

### Number of experiments

To obtain all the described variables 15 test days were used. All experiments were done in duplicate in the same room and under identical conditions.

### Test of bacterial growth

Trypsin soya agar (TSA) imprint plates were used for bacterial growth. The imprint plates are 20 $cm^2$ and collects thus 80% of the bacteria of each square. Imprints on test squares with $10^3$ of each bacteria were taken from each plate before UVC exposure to ensure spontaneous death of bacteria before starting the experiment. Imprints from all other test squares were taken after the UVC exposure time.

### Incubation and reading of TSA imprint plates

Imprint plates were incubated at 37°C. *C. difficile* was incubated anaerobic and the other bacteria aerobic. The number of bacteria (CFU) were counted after 72 hours.

### Field test

An outpatient clinic for patients with cystic fibrosis were selected for the field test. The natural bacteria flora in the room was used for measuring the changes in the number of bacteria in the room before and after disinfection. No external bacteria were added to the room.

Two patient's chairs, one patient table, one doctors table, and one door handle were selected as critical points where imprints with TSA imprint plates were taken before and after exposure to high doses UVC light (See technical notes).

## Results

### Laboratory test

Effect of UVC on vegetative bacteria after 15 minutes UVC exposure is shown in table 2a and 2b. The number is given as the average value of the two experiments. The highest start concentration of about $10^8$ CFU/cm$^2$ on the plates are shown in the first column. The next columns show the number of bacteria remaining on the plates at 3 meters, 4 meters and 5 meters from the UVC source.

| Bacteria | Highest concentration of bacteria on the plates before exposure to UVC (Average value) | Concentration at 3 meters after exposure to UVC (Average value) |
|---|---|---|
| *Staphylococcus aureus* | 6.9 x$10^8$ CFU/cm$^2$ | 1.2 x $10^2$ CFU/cm$^2$ |
| *Enterococcus faecalis* | 2.4 x $10^7$ CFU cm$^2$ | 1 x$10^2$ CFU/cm$^2$ |
| *Enterococcus faecium (VRE)* | 3.2 x $10^8$ CFU/ cm$^2$ | 5 x $10^2$ CFU/cm$^2$ |
| *Klebsiella pneumoniae* | 1.4 x $10^8$ CFU/ cm$^2$ | 0 CFU/cm$^2$ |
| *Acinetobacter baumannii* | 1.9 x $10^8$ CFU/ cm$^2$ | 0 CFU/cm$^2$ |
| *Stenotrophomonas maltophilia* | 3.6 x $10^7$ CFU/ cm$^2$ | 0 CFU/cm$^2$ |

***Table 2a***

| Bacteria | Concentration at 4 meters after exposure to UVC (Average value) | Concentration at 5 meters after exposure to UVC (Average value) |
|---|---|---|
| *Staphylococcus aureus* | 3 x $10^2$ CFU/cm$^2$ | 9.8 x $10^2$ CFU/cm$^2$ |
| *Enterococcus faecalis* | 1.3 x $10^3$ CFU/cm$^2$ | 2.4 x $10^3$ CFU/cm$^2$ |
| *Enterococcus faecium (VRE)* | 3.8 x $10^3$ CFU/cm$^2$ | 4 x $10^3$ CFU/cm$^2$ |
| *Klebsiella pneumoniae* | 1 x $10^2$ CFU/cm$^2$ | 7.5 x $10^1$ CFU/cm$^2$ |
| *Acinetobacter baumannii* | 5 x $10^1$ CFU/cm$^2$ | 5 x $10^1$ CFU/cm$^2$ |
| *Stenotrophomonas maltophilia* | 7.5 x $10^1$ CFU/cm$^2$ | 7.5 x $10^1$ CFU/cm$^2$ |

***Table 2b***

Gram negative bacteria were reduced about 8 $\log_{10}$ at 3m, 6 $\log_{10}$ at 4m and 6 $\log_{10}$ at 5m. A ten-fold reduction in the start concentration of bacteria resulted in a 7 log10 reduction at 5m.

Gram positive bacteria were less susceptible to UVC light than Gram negative bacteria. The reduction was around 6 $\log_{10}$ at 3m and around 5 $\log_{10}$ at 5m. A ten-fold reduction of the start concentration of bacteria resulted in 6 $\log_{10}$ reduction at 3 m and 5 $\log_{10}$ at 5m. Vancomycin resistant *Enterococcus faecium* seem to be less sensitive to UVC than other Gram-positive bacteria. The results of the effect of UVC light exposure for 15 minutes on vegetative bacteria are shown in table 2a and 2b.

Because of the high reduction of vegetative bacteria after 15 minutes exposure to UVC, the exposure time was reduced to 5 minutes at 2-meter 3 meter and 4 meters. All vegetative bacteria were reduced more than 5 $\log_{10}$ at 2 meter and more than 4 $\log_{10}$ at 4 meters after 5 minutes exposure to UVC (Figure 2).

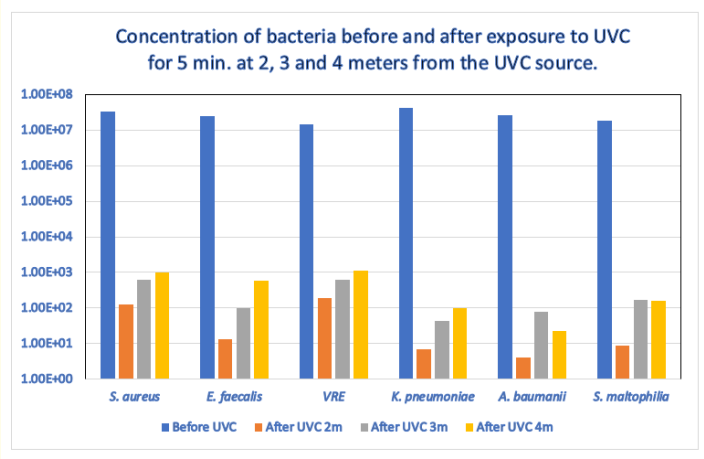


***Figure 2***

## Effect on spores

Effect of UVC on spores of *Bacillus cereus* after 90 minutes exposure was a reduction of 6 $\log_{10}$ at 2-meter, 3 meter and 4 meters from the UVC source. The reduction of *C. difficile* spores after 90 minutes UVC exposure at 2, 3 or 4 meters were about 1 $\log_{10.}$ Thus *C. difficile* spores are much less sensitive to UVC light than *B. cereus* spores (Figure 3).

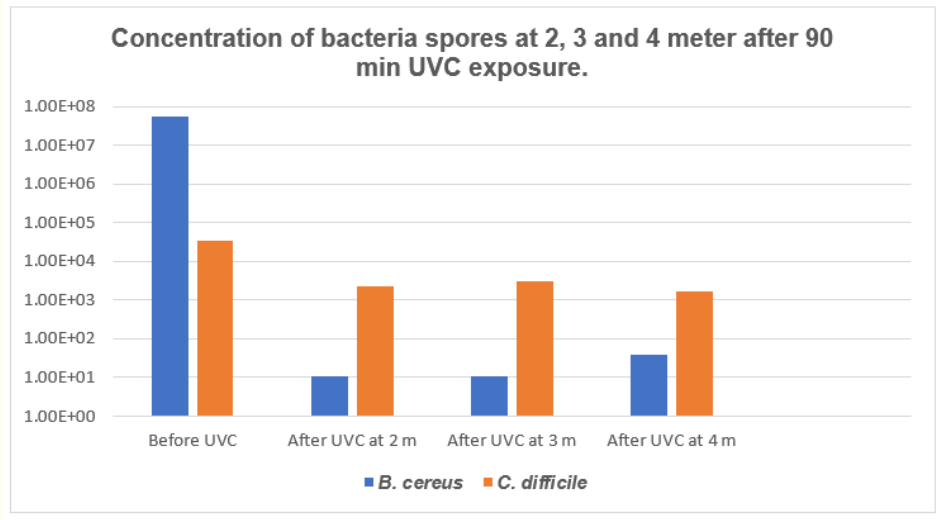


***Figure 3***

Effect of UVC on spores of *C. difficile* after 180 minutes exposure was a reduction of about 2.5 $\log_{10}$ at 2 meter and 1.5 $\log_{10}$ at 4 meters from the UVC source.

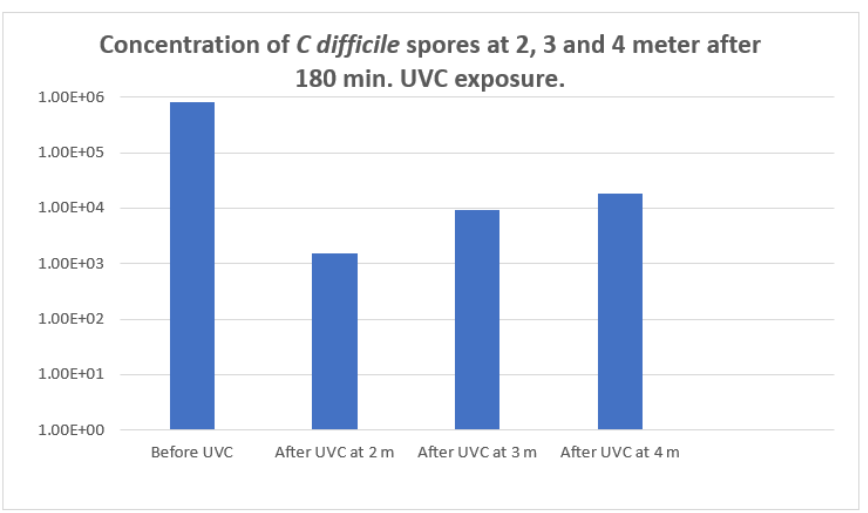


***Figure 4***

Vegetative bacteria are much more sensitive to UVC light than spores as expected. The longer exposure time to UVC the greater is the effect on both vegetative bacteria and spores and the closer the bacteria are to the UVC source the greater is the killing effect of UVC light.

### Field test

The field test was done in three rooms in an outpatient clinic for patients with cystic fibrosis. The UVC exposure time in-between imprints before and after exposure was 30 minutes. Imprints were taken from 5 standardized spots in each room. The results are shown in table 3. Samples were taken 8 times from room 1, 5 times from room 2 and 4 times from room 3. All numbers are given as CFU/imprint plate (20 $cm^2$) in table 3.

The results for each room are shown in figure 5a-5c. The bacteria distribution on each spot were the same before and after UVC but the number of bacteria was clearly reduced during the disinfection. Chairs were the most contaminated spots and here a clear reduction of bacteria were seen after UVC as shown in figure 6.

## Discussion and Conclusion

Several systems for room disinfection, hydrogen peroxide, peracetic acid, ultraviolet light and ozone has been introduces the last decades. For the use of hydrogen peroxide, peracetic acid and ozone it is necessary to seal the room to avoid leakage from the room. It may take an hour to seal a room which is not necessary when UV light is used. The disinfection time may depend on the system used, often between 30 and 90 min. After disinfection ventilation of the room is necessary for 60 - 120 minutes when hydrogen peroxide, peracetic acid or ozone is used but not when ultraviolet light is used. Thus, the total disinfection time is several hours shorter using ultraviolet light than with other disinfection systems. Therefore, this study is concentrated on the effect of ultraviolet light on microorganisms. Several systems using ultraviolet light has been introduced. In this study we examined the Dolphin Care room disinfection system.

| Spot | Room 1 (n = 8) | | Room 2 (n = 5) | | Room 3 (n = 4) | |
|---|---|---|---|---|---|---|
| | Before UVC | After UVC | Before UVC | After UVC | Before UVC | After UVC |
| Chair 1 | 12, 24, 36, 8, 2, 356, 3, 54 | 0, 0, 0, 0, 0, 6, 3, 0 | 0, 8, 21, 5, 62 | 0, 0, 9, 0, 0 | 18, 53, 288, 276 | 0, 0, 1, 2 |
| Chair 2 | 269, 57, 2, 3, 0, 119, 7, 34 | 3, 0, 0, 2, 1, 0, 2, 2 | 4, 9, 157, 2, 3 | 3, 0, 4, 0, 3 | 14, 348, 21, 47 | 0, 0, 1, 2 |
| Patients table | 9, 26, 3, 2, 5, 198, 6, 68 | 3, 0, 0, 0, 0, 0, 2, 0 | 15, 0, 4, 2, 3 | 0, 1, 10, 10, 2 | 11, 10, 81, 50 | 1, 0, 0, 5 |
| Work table | 5, 30, 17, 2, 1, 70, 6, 11 | 0, 0, 0, 1, 0, 0, 0, 0 | 3, 25, 24, 1, 21 | 5, 0, 0, 0, 1 | 8, 6, 26, 16 | 0, 0, 0, 0 |
| Door handle | 0, 3, 12, 1, 3, 52, 19, 6 | 1, 2, 0, 0, 3, 0, 1, 0 | 2, 1, 151, 0, 9 | 0, 0, 11, 0, 1 | 10, 2, 54, 16 | 8, 0, 0, 0 |
| Avarage | 39 | 1 | 22 | 2 | 63 | 1 |

***Table 3***

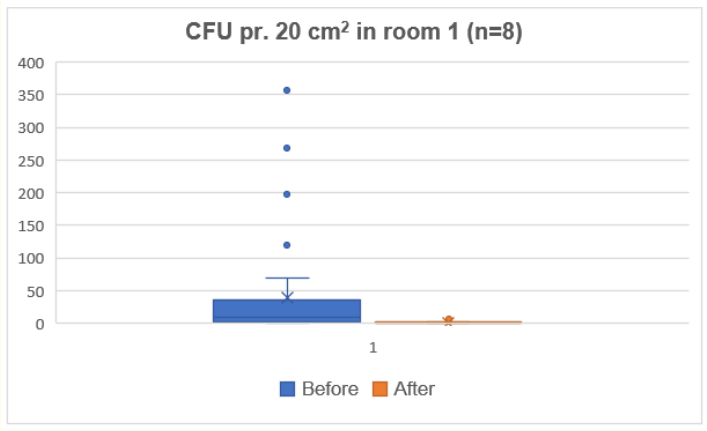


***Figure 5a***

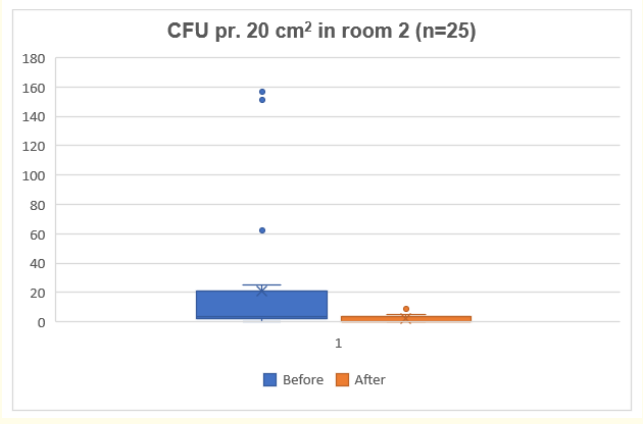


***Figure 5b***

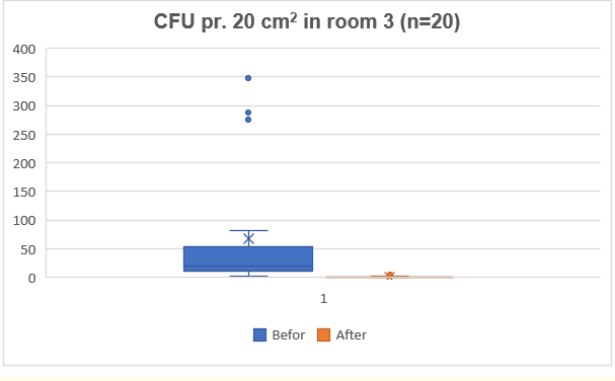


***Figure 5c***

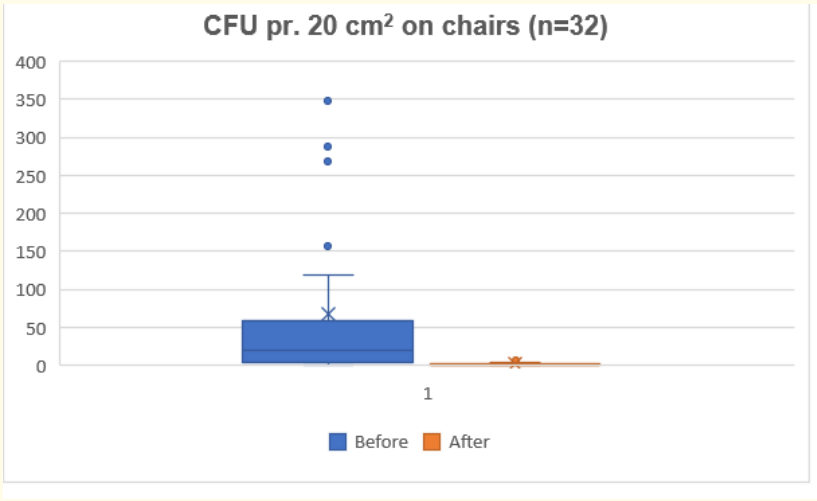


***Figure 6***

The effect of ultraviolet light on microorganisms was estimated both with laboratory tests and field tests. In the laboratory test known concentrations of vegetative bacteria and pure cultures of spores were used. The preparations were tested in different concentrations and at different distances from the UV light source. Vegetative bacteria were reduced 6-8 $\log_{10}$ at 3 meters from the UV source and 5-6 $\log_{10}$ at 5 meters. Thus, the disinfection with UV light is very effective even in rather large rooms of more than $50m^2$. Bacteria spores are less sensitive to UV light and only reduced 1-6 $\log_{10}$ after 90 min. *B. cereus* spores is more sensitive to UV light than *C. difficile* spores. This could be due to *B. cereus* having one membrane around the spore whereas *C. difficile* have two membranes around the spore.

The field test was done in an outpatient unit and the bacterial flora occurring in the rooms were used as indicator bacteria. This flora was dominated by coagulase negative staphylococci. In all three rooms there is a clear reduction in the number of bacteria on chairs and tables after disinfection with UV light. Chairs were the most contaminated spots that were measured and also here there was a clear reduction in the number of bacteria after disinfection with UV-light.

In conclusion, UVC light is an effective method, to kill bacteria in the laboratory, but also very effective to disinfect patients' rooms in short time.

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## Conflict of Interest

Leif Percival Andersen and Michael Tvede are advisor/consultant for Dolphin Care.

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