

Anti-microbial effect of filtered 222nm excimer lamps in a hospital waiting area

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Abstract

Hospital acquired infections is a considerable challenge for vulnerable patients. Traditional anti-microbial mercury lamps emit light at 254nm and have well established anti-microbial effects. Their use in populated areas is, however, hindered by their carcinogenic properties. This is in contrast to anti-microbial lamps based on krypton chloride (KrCl), demonstrated to have no carcinogenic potential. These lamps emit light with a peak intensity at 222nm and have broad bactericidal and viricidal effects including inactivation of SARS-CoVid-2 in laboratory experiments. Here, we investigate the bactericidal effects of filtered KrCl 222nm excimer lamps (UV222™ lamps) in an out-patient waiting area at the pulmonology clinic at Aarhus University Hospital. Furniture surfaces were sampled for bacterial load in a single-arm interventional longitudinal study with and without exposure to filtered 222nm UVC-light. Furthermore, bacterial species were identified using MALDI-ToF mass-spectrometry. We found filtered 222nm UVC-light to significantly reduced the number of colony-forming-units. Pathogenic bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis* were detected only in the non-exposed areas suggesting that these species in particular are highly sensitive to inactivation by 222nm UVC-light. This study demonstrates the potential use of 222nm Far-UVC technology to reduce bacterial load and thus the spread of infectious disease in a hospital setting.

Introduction

Hospital-acquired infections (HAI) is a major health problem on a global scale; it is estimated that 23.6% of hospital-treated sepsis cases are hospital acquired¹. In the USA alone, the applied cost for health care providers from HAI has been estimated to consist up to 14.9 billion USD in 2016², and furthermore, HAI account for up to 37,000 annual deaths in Europe and 99,000 annual deaths in the USA (WHO).

The recent Sars-CoVid-2 pandemic has highlighted our vulnerability toward spread of highly contagious infectious agents, and the importance of implementing and further developing techniques to prevent spread of contagions, especially in places where large numbers of persons pass by, or where particularly vulnerable persons gather. The clinic of pulmonology is an example of such a high-risk area. Here, numerous patients with respiratory diseases are crowded in a small area. Some patients visit the clinic to commence or follow-up treatment for infectious respiratory diseases such as complicated pneumonia, lung abscess, fungal pneumonia, tuberculosis or bronchiectasis and wait near other patients who are on immunosuppressive therapy, have had lung transplantation or have severely reduced lung function. Thus, spreading infection from one patient to another may have dramatic consequences in this population.

Use of anti-microbial measures such as frequent cleaning of rooms and furniture along with separation of patients and focus to reduce waiting time may lower risk for infections, but more efforts to modify the risk are needed and advanced techniques have evolved in recent years to supplement the effect of such measures.

Ultraviolet (UV) light is well-known to possess excellent disinfecting properties, by inducing the formation of photoproducts such as pyrimidine and 6-4 dimers in RNA and DNA, thereby interfering with transcription and replication³⁻⁵. “Classical” 254 nm UV light has long been used in biological safety cabinets in laboratories. However, the fact that conventional 254 nm UV light is highly carcinogenic in humans⁶ limits its potential for anti-septic utilization in locations with high risk of person-to-person transmission of contagions. Recently, a new generation of filtered excimer lamps based on excitation of krypton chloride (KrCl), generating 222 nm UVC-Light, has been introduced. In contrast to UVC-Light at 254nm, filtered 222nm excimer lamps (UV222™ lamps) can be safely installed in populated areas⁷⁻¹⁴.

In order to test the anti- microbial potential of filtered 222nm excimer lamps to reduce the risk of nosocomial spread of infections, we tested the effect of installing filtered 222nm excimer lamps in a out-patient clinic waiting room on bacterial load on exposed surfaces.

Results

Simulation of 222nm exposure from filtered 222nm UVC-lamps installed in the waiting area at Aarhus University Hospital

Using the simulation 3D-model of the waiting room (Fig. 1a) the overall exposure intensity (Fig. 1b) and the exact exposure on each chair (Fig. 1c) was calculated and the filtered 222nm excimer lamps were adjusted to deliver a total of 400 μJ for each on-cycle.

Effect of filtered 222nm UVC-Light

We found that filtered 222nm excimer lamps reduced the bacterial load of all chairs as the CFU count was significantly lowered from a mean of 26 CFU per sample without exposure to 8 CFU pr sample with exposure ($p < 0.0001$ by paired t-test)(Fig. 2A). Interestingly, we found that the installation of the filtered 222nm excimer lamps not only decreased the overall amount of CFU but also efficiently removed all high CFU counts (above 20 CFU per sample). The decrease in CFU was consistent when comparing the sampling days (Fig. 2B-D). Additionally, exposure to 222nm UVC-light was sufficient to decrease the CFU of both seat and backrest of all chairs indicating good coverage of the chairs with 222nm UVC-Light exposure (Fig. 2E-F). Furthermore, we found no pattern of variance throughout the sampling period thus suggesting that the sampling itself did not affect CFU numbers (Fig. 2G). Additionally, *in vitro* testing using Vero E6 TMPRSS2 cells and SARS-CoVid-2 showed high efficacy in viral neutralisation by filtered 222nm UVC-light (Supp. Fig 1a-c). Here, 10 seconds irradiation (approx. 520 uJ/cm^2) was sufficient to

reach a neutralisation of more than 50% while 2,5 minutes (7800 uJ/cm²) yielded a >90% neutralisation (Supp. Fig. 1a). With the high efficacy of filtered 222nm UVC-light, we investigated the effectiveness to decrease infectivity in an *ex vivo* model of the human airway epithelium. Here, we found 2 min. irradiation (6240 uJ/cm²) to decrease the amount of viral RNA in the cultures 40-fold (Supp. Fig. 1b). 5 min. irradiation (15600 uJ/cm²) decreased viral RNA by more than 10⁸-fold (Supp. Fig. 1c).

Bacterial load outside the exposed area

We also found a decrease in the bacterial load within the zone exposed to 222nm UVC-Light as compared to the chairs that were not exposed to the filtered 222nm excimer lamps (Fig. 3A). The reduction in CFU was consistent during all three sampling days yet with some variation in the degree of bacterial removal (Fig 3B-D). In accordance with results from the chairs that were exposed to the 222nm UVC-light, we observed that CFU were reduced on both the seat and the backseat of all chairs during the days with lamps turned on (Fig. 3E-F).

Identification of specific bacterial species using MALDI-TOF

MALDI-TOF MS identified multiple bacterial species. On non-UV chairs 17 different species were identified of which 10 were unique to these chairs as they were not found on exposed chairs. Interestingly, only 10 different species were found on chairs exposed to filtered 222nm UVC-Light, of which seven were seen on both non-UV and UV exposed chairs. (Table 1).

Discussion

This study aimed to evaluate the antimicrobial effect of filtered 222nm light and found that such exposure significantly reduces the overall bacterial load and seemed to eliminate a number of pathological bacterial species in a waiting area at a pulmonology out-patient clinic.

Conventional mercury UV light has long been known to have disinfecting capability but its carcinogenic potential limits use in areas populated with humans. Searches for a tolerable technique has led to the development of the excimer lamp that generates UV light at 222 nm, a wavelength safe to humans. Most studies published on the antimicrobial efficiency of UV at 222 nm have been performed in laboratory settings as proof of principle¹⁵. While results have been very positive with regards to both human safety

and in-vitro antimicrobial effect, this study is the first to show efficacy in a real-life setting and finds two interesting results.

Firstly, the ability of filtered 222nm excimer lamps to significantly decrease the CFU of hospital waiting room chairs highlight the usability of these types of devices in the clinical setting. Of specific interest is the ability of filtered 222nm excimer lamps to eliminate all higher CFU counts possibly stemming from patches of high bacterial density. It is reasonable to assume that these high-density patches potentiate a high-risk bacterial spread. Therefore, removal of all higher bacterial patches could potentially lower spread of bacteria from this surface. This would be of tremendous benefit as a pulmonology out-patient waiting room has a high flow of patients; some patients carry pathogenic and some patients carry antibiotic resistant bacteria and some patients are immunocompromised and may develop fatal illness if exposed to these bacterial patches.

Secondly, we show that filtered 222nm excimer lamps remove highly pathogenic bacterial species. Of specific interest is the removal of *Staphylococcus aureus* as this bacterial species is known for its potential to develop antibiotic resistance¹⁶. As both Methicilin Resistant and Methicilin Sensitive *Staphylococcus aureus* (MSSA and MRSA respectively) represent an increasing problem in hospital environments and nosocomial infections with these species are common and lead to increases in duration of hospital admission¹⁷. Thus, removal of this species using filtered 222nm excimer lamps could be of high value especially in departments treating patients with compromised immune systems who easily suffer from infections and in surgical departments. Also of interest is the apparent removal of *Staphylococcus epidermidis*, which is known to cause infection following insertion of catheters and is often resistant to therapy due to biofilm formation¹⁸. Reduction in biofilm-forming bacteria could be of huge benefit, not only in the clinical world but also in multiple industries suffering from the effects of bacteria in biofilms.

Finally, laboratory investigations confirm the already known effect of filtered 222nm UVC-light in neutralizing SARS-CoVid-2. Our study however, also highlight the end-result of this neutralization as irradiated virus are unable to establish a potent infection in the human airway epithelium. This, coupled with the highly decreased overall CFU and removal of high-density patches of bacteria showcase the potential of this technology in decreasing pathogen spread in areas with a high flow of patients.

One limitation of this study is that bacterial samples were only taken from surfaces; no air sampling was done. In patients suffering from respiratory diseases, inhaled bacteria are of special interest, however, it is reasonable to assume that free flowing microbes also are eliminated by 222nm UVC-Light. This hypothesis was tested by Eadie et. al., (2021), who found filtered 222nm UVC-light to efficiently inactivate aerosolized *Staphylococcus aureus*. The group reduced bacterial steady-state load with 92,1% as compared to a room without any 222nm UVC-light source, demonstrating the effectiveness of this technology in constant sterilization of air. Aerosolized particles are exposed from all sides and are more

easily eliminated than surface fixed bacteria and are also in closer proximity to the lamps that receiving a larger dose of 222nm light. A theoretical risk of exposure could be that elimination of some bacteria allow other species to develop, and if these are pathogenic, the filtered 222nm excimer lamps would potentially create a more dangerous milieu. However, results from the MALDI-ToF rejects this concern. Importantly, the primary study objective was to reduce bacterial load in a hospital environment to potentially reduce the risk of acquiring HAI's. A significant reduction was achieved but whether this results in fewer HAI's in patients is yet to be studied.

Finally, following the SARS-CoVid-2 pandemic, it has become evident there is a need for better and more efficient disinfection techniques in areas with high density of people. Especially, techniques targeting not only viral pathogens such as SARS-CoVid-2 but all disease-causing microorganisms are highly sought after. We did not include SARS-CoVid-2 testing of the waiting area in our study as we expected very low, if any, of this virus in the out-patient clinic. This due to all patients testing negative before visits. However, viruses, including SARS-CoVid-2 are more easily eliminated by 222nm UVC-Light than bacteria¹⁵ and is therefore reasonable to assume that filtered 222nm excimer lamps would inactivate these viruses in this setting as well. A limitation to 222nm UVC-Light technology is that it only exert its effect on surfaces that are exposed and thus not on shadowed areas. Although UVC-Light is reflected and thus will reach most surfaces, those surfaces that are only indirectly exposed are likely to require much longer exposure time to experience significant reductions in bacterial load. In conclusion, this study shows the usability of filtered 222nm excimer lamps in a patient waiting area. These devices, being safe for use in areas occupied by humans, have a high potential to become the disinfecting technology of the future as they can generate a continuous anti-microbial environment in areas with high density and high flow-through of people.

Methods

Aims, setting and study design

The primary aim of this study was to investigate the anti-microbial potential of filtered 222nm excimer lamps in an out-patient hospital setting. A prospective longitudinal single arm interventional study with serial sampling was designed and set up in the waiting area at the out-patient clinic at the Department of Respiratory Diseases and Allergy, Aarhus University Hospital, Aarhus, Denmark.

Secondary aims were to evaluate how the filtered 222nm excimer lamps affected bacterial load in the 222nm exposed area compared to chairs placed in the more distant area of the waiting area and thus outside the range of the filtered 222nm excimer lamps. Furthermore, we aimed to identify the bacteria load and determine which bacterial species were present on non-UV-exposed chairs compared to 222nm-exposed chairs.

The waiting area

In the clinic's waiting area, patients await consultation or respiratory examinations. The clinic has services for patients with complicated infectious respiratory diseases, follow up after lung transplantation, severe asthma, interstitial pneumonias, and suspected lung cancer and is equipped with 10 chairs.

Filtered 222nm excimer lamp

The far-UVC source used in this study was a germicidal lamp (UV222™, UVmedico, Denmark) based on a filtered KrCl* excimer light source emitting at 222 nm (Care222, Ushio, Japan). The optical filter blocked the remnants in the 230-350 nm emission range which are naturally present in KrCl* excimer lamps. At 222 nm the lamp had a total output of 120 mW, with a full-width half-max emission angle of 60 degrees, resulting in an irradiance of 13.7 $\mu\text{W}/\text{cm}^2$ at 1 m distance. The output power, optical spectrum and spatial distribution of the lamp has been characterized using a UV calibrated goniometer ("LabSpion" from "Viso Systems"). Input information into the UV222™ software contained data on distance to nearest unprotected eye, maximum occupancy time per patient or staff, and dose delivered (dd) per on-off cycle in the farthest distance from the lamp. In this instance, dd for chair seats were 400 μJ , which is the approximate equivalence of the dose needed to reduce infectivity of SARS-CoVid-2 approximately 90%^{7,15}.

Simulation of waiting room

For simulation and visualisation of the light distribution and energy levels on different surfaces in a room, the DiaLux EVO version 9.2 Light simulation software was used. The fixture files used in the program were verified and measured using the reference LabSpion system and goniometer from VisoSystems. To illustrate the setup and to calculate 222nm doses, a 3D-model of the waiting area and the placement of the lamps was generated (Fig. 1a). Evaluation of the overall exposure intensity (Fig. 1b) and the exact delivered energy in $\mu\text{W}/\text{cm}^2$ on each section of the chairs (Fig. 1c) was done using this model. Based on the energy values on the outer part of the two chairs positioned under one of the lamps (1,3-2,0 $\mu\text{W}/\text{cm}^2$), we adjusted the filtered 222nm excimer lamps to deliver a total of 400 μJ for each on-cycle. The rationale for this dosage is that many viruses including SARS-CoVid-2 are significantly inactivated at this dosage¹⁵. Infectious viruses are present mainly in aerosols in the air and thus closer to the lamp. As the

intensity of the emitted light increases with increased proximity to the filtered 222nm excimer lamps, viruses suspended in the air would then receive a sufficient 222nm dose to significantly reduce the risk of transmission in one on-cycle^{7,15}. The length of the off-cycle was adjusted so that patients were never exposed to more than 22.3mJ in one visit, which is maximum daily exposure limit set by the Danish government (<https://at.dk/media/1947/562bilag2pdf.pdf>) and the European Union (<https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006L0025-20140101&from=DA>).

Bacterial load sampling

Bacterial loads were determined using Hygicult® TPC sampling kit (#510068010, Food Diagnostics, Grenaa, Denmark) in accordance with the protocol supplied by the manufacturer. The agar-covered sampling sticks were used to collect samples from chair seats and backrests. For each seat or backrest sampling, two measurements were performed using the two sides of the Hygicult sampling stick. The sampling sticks were then incubated overnight at 38 deg C before counting bacterial colonies.

Samples were collected at the same time in the afternoon on three consecutive days with the filtered 222nm excimer lamps turned off and repeated for three consecutive days with the lamps turned on. As the same spots were sampled on each chair on three consecutive days, CFU counts on each day were assessed to see if variance in bacterial load was introduced.

Maldi-Tof MS identification of bacteria

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), was used to determine which bacterial species were present on non-UV-exposed chairs compared to 222nm-exposed chairs. Hygicult® TPC sampling sticks were randomly selected and analyzed.

SARS-CoVid-2 TCID50% assay

Assay performed as previously described in Olagnier 2020¹⁹. In short, DMEM containing 6.76×10^6 pfu SARS-CoVid-2 (strain #291.3 FR-4286) were either untreated or exposed to UV222. Each viral sample were

then serially diluted in plates containing Vero E6 TMPRSS2 cells and investigated for cytopathogenic effects after 72 hours.

Air-Liquid interface (ALI) epithelium model

Cell model performed as previously described in Olgagnier 2020¹⁹. In short, ALI cultures were infected using DMEM containing 6.76×10^6 pfu SARS-CoVid-2 (strain #291.3 FR-4286) either untreated or exposed to UV222. ALI cultures were exposed for one hour before the DMEM was removed and cells were incubated until harvest.

qPCR analysis

Assay performed as previously described in Olgagnier 2020¹⁹. In short, RNA was isolated from ALI cultures and tested for ACTB (Taqman, Hs01060665) and SARS-CoVid-2. SARS-CoVid-2 primers and probes used were: Forward primer; AAATTTTGGGGACCAGGAAC, Reverse primer; TGGCACCTGTGTAGGTCAAC, and probe FAM-ATGTCGCGCATTGGCATGGA-BHQ.

Statistical analysis

Paired student's t-test were performed according to analysis of parametric data distribution. A student's t-test was done to evaluate change in bacterial load over the days of sampling. Statistical level of significance was set to 5%. Data were analysed in GraphPad Prism.

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Declarations

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Author contributions:

J.T., S.H.S., A.F.H., E.B., P.H., and C.K.H. conceived the project. J.T., S.M.J., S.Y.N., and C.K.H planned and performed experiments. J.T. and C.K.H. analyzed data and created figures. J.T., S.H.S., A.F.H., E.B., P.H., and C.K.H drafted the manuscript. J.T., and C.K.H., finalized manuscript and prepared for publication.

Competing interests:

Christian Kanstrup Holm is co-owner and Jacob Thyrsted is part-time employed at UVmedico, which is a manufacturer of UV222TM nm lamps. Søren Helbo Skaarup, Andreas Fløe Hvass, Sara Moeslund Joensen, Stine Yde Nielsen, Elisabeth Bendstrup and Pernille Hauschildt declare no potential conflict of interest.

Data availability:

All data generated or analysed during this study are included in this published article.

Ethics declaration:

Per local regulations, informed patient consent was not required, as no sensitive data were recorded. The filtered 222nm excimer lamp is commercially available (UV222™) and approved by Conformité Européenne, the CE-mark, meeting European Union standards for health and safety. Conduction of the study was approved by the chief of department.

Tables

Table 1 is in the supplementary files section.

Figures

Figure 1

Installation of filtered 222nm excimer lamps

Depiction of how filtered 222nm excimer lamps (UV222™ lamps) were installed in a section of the waiting areas at Aarhus University Hospital, Department of Respiratory Diseases and Allergy. A) 3D simulation of the waiting area covered by the UV222™ lamps. B) Depiction of 222nm emission. C) Simulation of surface dosage of 222nm in μW .

Figure 2

Filtered 222nm excimer lamps reduce bacterial load in hospital waiting area

Filtered 222nm excimer lamps lamps placed as indicated in figure 1. Bacterial loads were estimated by sampling of seat and backrest of chairs placed in the patient waiting area. Sampling was performed on three individual days from three individual chairs before installation of the UV222 lamps (No UV) and then again on three individual days on the exact same chairs after exposure to 222nm UVC-Light. Bacterial samples were collected using Hygicult TPC sampling kits. Bacterial colonies were counted after overnight incubation at 38 deg C. UV222 lamps were installed with software to secure that no patients and no staff were exposed to 222nm doses exceeding 22mJ per working day. Bacterial colonies are depicted as total number of colonies A), each individual day B-D), colonies on seats E) and on backrest

F). To determine if sampling in itself affected bacterial load we compare the counts between days from the No UV chairs G).

Statistical analysis was performed using paired students t-test and p values are depicted together with each figure panel. Bars indicate mean +/- s.e.m and each dot represents one biological sample.

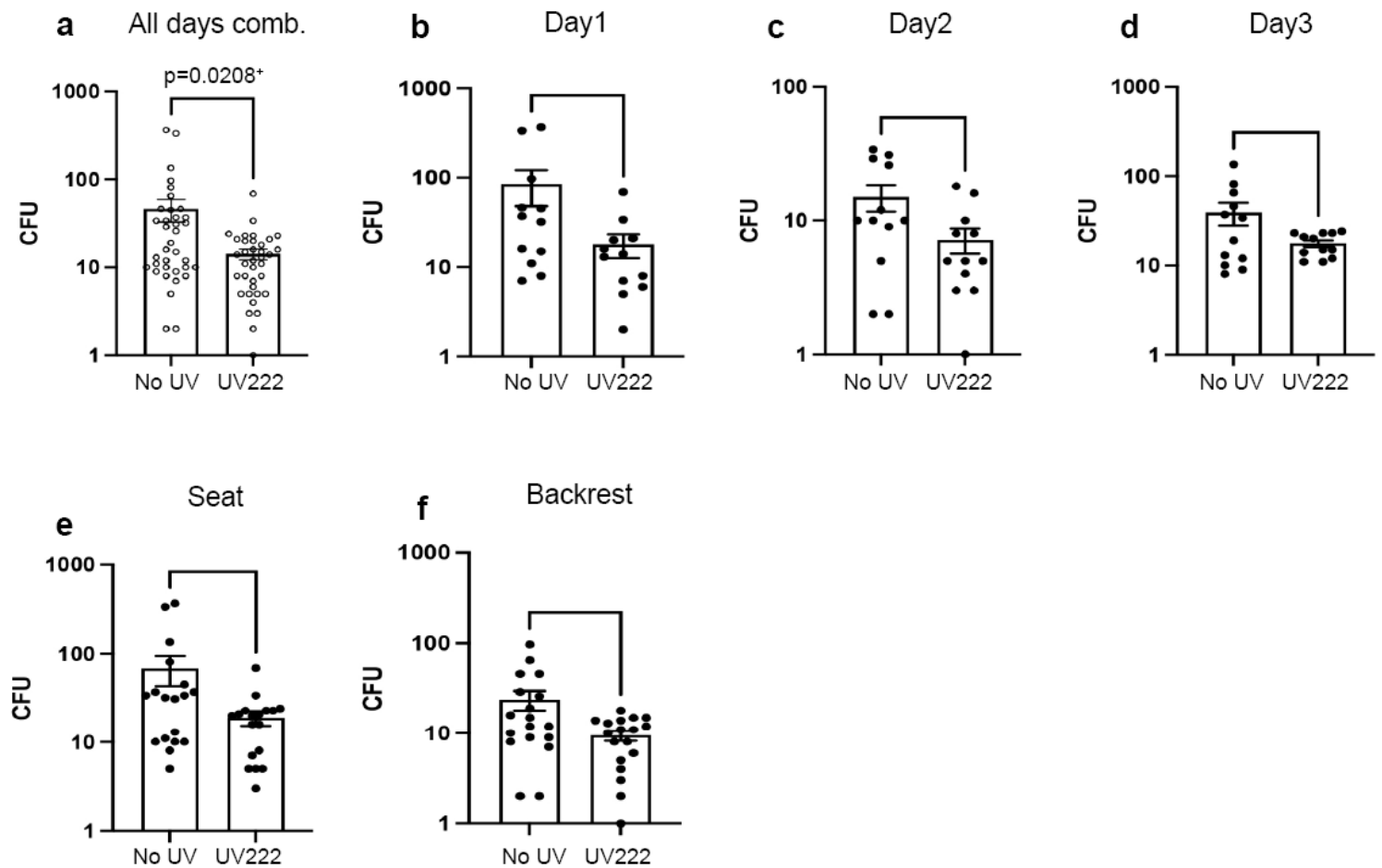


Figure 3

Filtered 222nm excimer lamps reduce bacterial load in hospital waiting area, part two

Filtered 222nm excimer lamps were installed as indicated in figure 1. Bacterial loads were estimated by sampling of seat and backrest of the chairs placed in the patient waiting area. Sampling was performed on three consecutive days from three individual chairs placed within the UV exposed area (UV222) and from three individual chairs placed outside the UV exposed area (No UV). Bacterial samples were collected using Hygicult TPC sampling kits. Bacterial colonies were counted after overnight incubation at 38 deg C. Bacterial colonies are depicted as total number of colonies A), each individual day B-D), colonies on seats E) and on backrest F). Statistical analysis was performed using unpaired students t-test (+) and/or Mann-Whitney (*) and p values are depicted together with each figure panel. Bars indicate mean +/- s.e.m and each dot represents one biological sample.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.pdf](#)
- [Supp.Fig.1.pdf](#)