Author's response to reviews

Title: Evaluation of an automated ultraviolet radiation device for decontamination of Clostridium difficile and other healthcare-associated pathogens in hospital rooms

Authors:

Michelle M Nerandzic (michellenerandzi@aim.com)
Jennifer L Cadnum (cadnumil@gmail.com)
Michael J Pultz (michaelpultz@gmail.com)
Curtis J Donskey (curtisd123@yahoo.com)

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Author's response to reviews: see over
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Dear Editor,
We would like to submit a revised version of our manuscript entitled "Evaluation of an automated ultraviolet radiation device for decontamination of *Clostridium difficile* and other healthcare-associated pathogens in hospital rooms" for consideration as a Research Article in *BioMed Central Infectious Diseases*. We appreciate the helpful comments of the reviewers and Associate Editor. We have modified the manuscript accordingly and believe that it is significantly improved. Our responses to the comments are below.

Thank you for your consideration,

Curtis Donskey, M.D.
Cleveland VA Medical Center, Geriatric Research, Education and Clinical Center, 10701 East Blvd., Cleveland, Ohio 44106
Phone: 216-791-3800 ext. 6153 or 4788
Email: curtisd123@yahoo.com
In response to the comments of the Associate Editor:

As raised by two of the reviewers, some important methodological issues have not been adequately described. The authors should go through those issues raised by reviewer #1 and #3 carefully and address their concerns in the revision. It is not clear how many patient rooms were sampled after they were vacated by patients. The number of samples taken for each room and how they standardized the areas to be sampled also required clarification.

We have clarified these issues as noted below in our responses to the reviewers.

We have included information on the number of rooms sampled after they were vacated by patients (66 rooms) on lines 128 and 208. We have more clearly stated the number of MRSA rooms (26 rooms) cultured before and after Tru-D disinfection (lines 48 and 221). The number of samples taken (4 per room) is stated on line 131.

Reviewer 1 raised one very important question ? i.e. how did the authors know all the surfaces in the patient room were exposed to the intended doses. If not, then the surfaces that were shaded will remain contaminated. Unlike vapour or fume disinfection, the UV light is not expected to penetrate every item surface in the room. All this go back to the number of sites sampled and how you choose them. If only the well irradiated surfaces were sampled, you have a marked reduction as was observed in the laboratory experiments. However, if you sample more sites/room and include those areas in the patient room that may not have sufficient exposure to the UV light, then the result could be totally different.

We have added additional information to clarify how the device works (Methods, lines 91-106). UV-C radiation penetrates all areas of the room that receive light; areas that are not in direct line of exposure to the output of the device may receive radiation that is reflected from the walls and ceiling or from other surfaces in the room. The device has 8 sensors that measure the amount of UV-C radiation reflected back to the device. The cycle automatically ends when the area of the room reflecting the lowest level of UV-C back to the sensors (i.e., shaded areas) has received an adequate dose. Therefore, although it is true that the corners of the room or shaded areas receive a UV-C dose that is lower than for the areas directly adjacent to the device, all areas that are exposed to light will receive UV-C and the device is calibrated to provide an adequate dose to the area of the room reflecting the lowest level of UV-C.

To better address the question of how effective the UV-C device is in areas of patient rooms that receive lower radiation doses, we performed an additional experiment (Methods, lines 138-146); reduction of spores inoculated onto plastic carriers was compared at 20 inches versus at the farthest corner of the room which was 10 feet from the device and not in direct line of exposure to the output of the device (i.e., placed behind the bed so UV-C exposure was indirect). As we have noted in the Results (lines 216-220), there was a significant reduction in recovery of spores at the indirect corner site, but the reduction was significantly less than was observed at 20 inches from the device. In the Discussion (lines 275-279), we have noted that this is a potential limitation of the device.
In response to the comments of Reviewer 1:
1. Line 95; how can surfaces be selectively treated by the device? Is this managed by positioning? Given the nature of the disinfectant vapour devices currently on the market, room disinfection is usually achieved by positioning the robot in the middle of the area to be decontaminated and the room sealed off. It is presumed that all superficial surfaces are exposed to the disinfectant fumes in the room. Is this not the case for this device?

As noted previously, we have added additional information to clarify how the device works (Methods, lines 91-106). The device does not selectively treat certain surfaces. Rather, UV radiation penetrates all areas of the room that receive light; areas that are not in direct line of exposure to the output of the device may receive radiation that is reflected from the walls and ceiling or from other surfaces in the room.

2. Line 97; no information on density of original inoculation onto surfaces, ie. cfu/cm². Similarly, the surface area sampled by swabs taken after irradiation is not detailed.

We have added more information on the surface areas sampled. For surfaces artificially inoculated with organisms, the surface areas tested were 1 cm² (lines 109-110 and 141-142). For sites in hospital rooms cultured for environmental contamination (Methods, lines 132-143), the results were re-calculated and expressed in \( \log_{10} \) CFU/cm² or mean CFU/cm² (lines 190-194) and figures 2-4.

3. Line 105; generally speaking, this sort of sampling is repeated in triplicate for in-vitro experiments, especially when disinfectant activity is being assessed.

A 3rd set of experiments was performed and the figure (Figure 2) was adjusted to include the additional data. We have noted that the experiments were performed in triplicate (line 122).

4. Line 108; it is entirely appropriate to specify the areas sampled in rooms of discharged patients but how was this determined? Was it guesswork or was a template of some sort utilized? An approximate value for the surface area of the call button and telephone should be included.

The surface areas to be sampled were measured and the same standard surface area was sampled each time. We have not modified the manuscript since it is already stated that a 5 x 20 cm area was sampled. We have added an approximate area sampled for the call button and telephone (line 136).

5. Line 121; what was the inoculum of Staphylococcus warneri placed on additional surfaces? Density of inoculum on the surface per cm²? Was it standardised between different sites? Knowledge of the absolute areas of treated surfaces is important for several reasons, but particularly so when the output of the device itself is measured quantitatively in cm².

We have added the requested information on the inoculum of S. warneri (lines 154-167). Surface areas were standardized to 1cm² as described in response to comment 2 above.

6. No data given in the methods section on the total number of sites sampled. It is not actually clear in the methods whether the clinical environmental sites were inoculated with test pathogens (presumably not) or whether the investigators assumed that there would be plenty of sites positive for the chosen pathogens, depending upon which patient rooms were selected for testing. Were rooms
chosen on the basis of prior occupants being positive for one or more of the pathogens of interest, other than MRSA?

The total number of rooms (N = 66) and sites (N = 261) cultured has been added to the Methods section (line 128 and 137) and is noted in the Results section (line 208). We have noted that the clinical sites were not inoculated with test pathogens (lines 129-130). We have noted that rooms were chosen based on prior occupancy by patients on contact precautions for MRSA or C. difficile (lines 130-131).

7. Line 171; given the lack of data on areas sampled for some sites, I feel somewhat uneasy at the statement regarding the reduction of cfu’s following device exposure. If the sampling had been rigorously standardised, then the investigators would be able to more accurately titrate the effect of the UV-device.

As noted above, we have included additional information on the areas samples and have standardized all of the results to CFU/cm\(^2\) as requested (Figures 2-4).

8. Line 179; How was S.warneri identified from surface samples after routine cleaning and exposure to the UV device? How does the team know that it was the same organism as planted previously? Could post-treatment samples not have been contaminated with other strains of S.warneri, or indeed, other types of coagulase-negative staphylococci? Similarly, given the lack of data on original inoculation and areas sampled, any comments regarding reduction of growth density are not justified.

We have included information on the original inoculation amount (CFU/cm\(^2\)) and areas sampled (lines 154 to 167). We did not perform molecular typing to ensure that the S. warneri recovered from surfaces was identical to the organism planted. However, the sites were disinfected with bleach prior to inoculation to eliminate any pre-existing contamination and control sites that were not inoculated with S. warneri were negative for contamination post-treatment. In addition, for the comparison of recovery of S. warneri after UV-C disinfection or routine hospital cleaning, any potential contamination with environmental coagulase-negative staphylococci would not alter our conclusions since it would have been equally likely for the standard cleaning and UV-C surfaces.

9. There are too many Figures, most of which are not needed to demonstrate overall effect of the UV-device. I would suggest choosing one or two at most to illustrate the log reduction of organisms before and after exposure.

We have removed figures 2 and 5.

A few minor points:

1. Enterococcus should not be italicized; lines 27, 45

We have not made the requested modification (lines 28 and 48) because we believe that it is correct to italicize the genus name *Enterococcus* (i.e., vancomycin-resistant *Enterococcus* but not vancomycin-resistant enterococci) and have done so in many previously published articles.

2. Five figures but no photograph or illustration of the device itself. Perhaps this could be included provided no legal caveat?

A photograph of the device was added (Figure 1).

3. The report is over referenced, particularly on papers about the effects of UV light.
We have not removed references. Since there are no previous publications on killing of C. difficile by UV-C, we believe that it is appropriate to cite several references regarding killing of various types of bacterial spores by UV-C.

4. How big is the device? Is it portable? Comment on purchase cost?

We have added a description of the device (lines 91-106) and a picture (Figure 1).

We do not have information on the purchase cost.

Conclusion

It is certainly nice to see innovative attempts at assessing environmental cleaning in hospitals, but this study needs considerable tightening on its methods of quantitative sampling. In addition, seeding sites with coagulase-negative staphylococci in the hospital environment might actually arouse ethical interest, since no data is given on the probity of the strain used. Previous studies have used pieces of virus that are non-active, followed by molecular methods to confirm persistence; others have used genotyping to confirm identity.

As noted previously, we have modified the Methods and Results sections to present the results in a more quantitative manner. We have added information on the coagulase-negative organism that was used (lines 84-85). Coagulase-negative staphylococci are relatively common environmental contaminants in our facility, but there have been no previous infections with S. warneri identified in the past 10 years. The test strain was susceptible to multiple antibiotics including penicillin. Since our goal was to assess the ability of the UV-C device to reduce levels of live bacteria, use of non-active pieces of virus would not have been appropriate for our study. As noted previously, we did not perform molecular typing to confirm that the recovered organism was S. warnerii, but negative control sites that were not inoculated were negative for coagulase-negative staphylococci (lines 165-168). Finally, all sites that were inoculated with S. warneri were thoroughly disinfected with 10% hypochlorite solution after sampling was completed (lines 168-170).

In response to the comments of Reviewer 2:

1) Major Compulsory Revisions: None
2) Minor Essential Revisions: Authors should state whether or not they think that paired statistical analyses are more appropriate for analyzing results obtained from the same surface before and after disinfection.

See comments below.

3) Discretionary Revisions: Consider citing additional unpublished study on the Tru-D system.

As noted below, we have cited the unpublished study.

General comments:

8) On pages 8 and 9, when the proportion of specified surfaces contaminated before and after disinfection was performed, shouldn't paired analysis have been performed, since the same surfaces were cultured at the same sites? If so, McNemar’s test and paired T tests would seem to be appropriate. Nonetheless, I doubt that a change in statistical methods would have altered the authors’ conclusions.
We mistakenly stated that unpaired t-tests were used when we actually used paired t-tests. We have corrected the text to state that paired t-tests were used (line 189).

9) On page 9, lines 190-191. Did the investigators or housekeepers notice any unusual odor in the rooms when the door was opened at the end of the disinfection cycle? If so, how did housekeepers react to any such odor?

The investigators and housekeepers noted that there was an odor when the door was opened at the end of the disinfection cycle. However, the odor quickly dissipated and no healthcare workers or patients commented about the odor. This description was added in the Results section (lines 239-242).

12) Discussion of this type of area decontamination and other methodologies reported in the literature is appropriate. The authors might consider citing the unpublished study by WA Rutala, presented in June 2009, and incorporated in a Powerpoint presentation available on the Disinfection and Sterilization website/Martin S. Favero Lectureship:


We have briefly noted in the Discussion that our findings are consistent with the unpublished findings of Dr. Rutala and cited a recent presentation of the data at the 2010 Society for Healthcare Epidemiology of America meeting.

In response to the comments of Reviewer 3:
- Major Compulsory Revisions

Lines 92 to 105: This section needs major revision and additional detail to clearly establish what was done. Were swabs used to examine the level of artificial contamination applied to the bench top and this amount was then adjusted to log 3 – log 5 cells before dosing with UV? If the inoculum was adjust to log 3 – 5 before inoculation, how was this achieved? Over what surface area was the inoculum applied? How can direct plating onto agar plates from a swab be used to assess if there are 10,000 cells inoculated onto a surface? Over the time course between swabbing and determining how many bacteria have been inoculated onto a surface, will the amount of culturable bacteria be naturally reduced or not?

We have revised this section to include the requested information (lines 105-118).
- Minor Essential Revisions

Line 30, 31: Suggest that “logs” be revised to log10 2-3 CFU cm-2 or m-3 or scientific notation as appropriate. Recommend this be used throughout the manuscript and any figures so that continuity is maintained

We have made this change as recommended throughout the manuscript.

Line 34: change to: “After routine cleaning of the rooms of MRSA carriers, …..”

This correction was made as suggested (lines 35-36).

Line 91: Eradication of pathogens inoculated onto surfaces – there was a reduction in the amount of organisms not a complete eradication from what I can see. Revise here and throughout

We have changed eradication to reduction throughout the manuscript (line 91).

Line 97: Pre-moistened swabs – pre-moistened with what and what kind of swabs (manufacturer??)
We have added the brand name and manufacturer (lines 119-120). The swabs were pre-moistened by the manufacturer. Line 98: “For each pathogen, the inoculum applied...” Should be moved to earlier in the paragraph "For each pathogen, the inoculum applied..." was moved to lines 111-112 earlier in the paragraph. 

Line 99: use either log 3 – log 5 CFU or 10^3 to 10^5 throughout the manuscript for continuity 

The manuscript was standardized to log_{10} CFU/cm^2 for continuity (line x). Lines 111-113: Where the phrase “cultured” is used, the reader should be referred to the section that explains how this was done i.e. as described above OR as described below etc

We have referred readers to the Microbiology section of the methods (lines 132-133). Line 121: Staphylococcus warneri was also inoculated... - please indicate over what size surface area and the concentration of the inoculum that was used. Also in line 125 if not the same. How was this prepared and what media was then used to culture the organism, this is not indicated in the manuscript. 

We have added the requested information (lines 147-153 and lines 170-171). Lines 150 – 162: Figure 1 shows the average log reduction of various organisms after exposure. However it would possibly be better to know exactly how much was inoculated in each instance. For example if there was log10 3 CFU cm-2 inoculated and this was reduced by log10 2.5 CFU cm-2 the effect is obviously greater than if there was log10 5 CFU cm-2 inoculated and log10 2.5 CFU cm-2 remains on the surface. If possible, substituting Fig. 1 for a detailed table of inoculation levels and then the amount recovered after exposure would give the reader a better indication of the effectiveness of the device. Suggest a revision of this section. 

For these experiments, the reduction was measured by comparing the CFU recovered before versus after treatment. Since there was some variability in the initial inoculum and there was a large sample number (N = 42), we believe that a detailed table would be less effective at conveying the results than a concise figure showing average log reductions. Error bars were included to demonstrate that there was little deviation among the average reductions whether the original inoculum was log_{10} 5 CFU/cm^2 or log_{10} 3 CFU/cm^2. Figure 2 adds little to the overall manuscript, suggest removal. Likewise figure 5. 

Figures 2 and 5 were omitted from the manuscript.

Lines 186-187. Difficult to gauge the effect of disinfection as the level of S. warneri inoculation is not stated in the methods. 

The S. warneri inoculum has been clarified (lines x to x). Line 217; I disagree with the authors; if the cycle requires only 45 minutes, this would be considered a rapid turnaround time in comparison with a method such as VHP. 

We have contrasted the cycle time for the UV-C device (45 minutes) with the time required for hydrogen peroxide vapor (several hours) (lines 252 and 256). Line 251: Please disclose if the system was provided at cost or free for the purpose of the study. 

The device was provided free of charge by the company until completion of the study. This study was not funded by the manufacturers of the UV system and the
company did not contribute to the design or writing of the study. This was more clearly elaborated on in the Acknowledgements section on lines 289-293.

Line 289: Reference should read: “Spores of Bacillus subtilis: their resistance to and killing by radiation, heat and chemicals”

Reference was corrected (line 331).

Discretionary Revisions
Introduction:
Can the authors indicate if the device needs to be moved or aimed manually at a surface or is this completely automated with the entire room receiving an adequate dose of UV without any manual interference? What about shaded areas?

We have added the requested information (lines 90-102).

Line 27: not sure commonly-touched needs hyphenation

Hyphen removed on line 28 of the abstract.

Line 79: suggest change to “… CDI in Cleveland were used in this study.”

Correction was made on line 78.

In response to the comments of Reviewer 4:

Major Compulsory Revision:
Given the nature of the study it would appear likely that it was supported by the commercial manufacturers of the UV system. Funding source disclosure should be noted.

We have noted in the Acknowledgements section (lines 289-293) that the study was not funded by the manufacturers of the UV system and the company did not contribute to the design or writing of the study. The devices were provided free of charge for the duration of the experiments.

Other Recommendations:
1. Consideration should be given to eliminating Figure 2. The figure photographically illustrates the effectiveness of the UV-C system in reducing the tested pathogens seeded onto a clean non-porous surface in the laboratory setting. Although substantial reduction in environmental contaminations was realized in the clinical setting, the impact was not to the level found in the laboratory setting. For this reason, this reviewer would suggest that Figure 2 is both not clinically relevant and has the potential for suggesting similar efficacy in the clinical setting.

Figure 2 was omitted from the manuscript.

2. P3L49 While the references cited are completely appropriate to the introductory statement being made, the most recent reference noted by the authors was published in 2008. This portion of the Introduction would be enhanced by including the additional relevant references published since 2008.

A more current reference was substituted (lines 323-325).