Author's response to reviews

Title: Evaluation of a hand-held far-ultraviolet radiation device for decontamination of Clostridium difficile and other healthcare-associated pathogens

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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 a hand-held far-ultraviolet radiation device for decontamination of *Clostridium difficile* and other healthcare-associated pathogens" for consideration as a Research Article in *BioMed Central Infectious Diseases*. We appreciate the helpful comments of the reviewers. We have modified the manuscript accordingly. Our responses to the comments are below.

Thank you for your consideration,

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In response to the comments of Reviewer John Boyce:

1. Page 6, lines 113-115 and page 10, lines 188-189. It was not entirely clear to this reviewer whether ozone measurements were conducted by the investigators, and if so, what methods were used.

   Ozone measurements were not conducted in this study; here we tested the manufacturer's claim of concomitant ozone production while administering far-UV irradiation on the device's "high power" setting. The manufacturer's describe the ozone production as "deodorizing" as opposed to disinfecting, however we wanted to determine whether the levels of ozone produced at this setting would enhance killing of pathogens on surface when combined with far-UV. As described, the low power setting does not produce ozone, yet achieves the same radiant dose of far-UV (albeit over a longer application period) as the high power setting. We found that there was no significant difference in the lethal effects of the device with equivalent doses of far-UV administered by the low power or high power setting. Therefore the ozone generated at the high power setting made no contribution to the device's disinfection capacity. In order to clarify our objective in this study a description of our intention was added to page 7 on lines 126-130 in the Methods section.

2. Page 11, lines 223-225. The text states that UV-C room devices may require 45 min to disinfect a room, and that hydrogen peroxide vapor (or mist) systems may require hours for disinfection of a hospital room. The study by Boyce et al. of hydrogen peroxide vapor did not evaluate various cycle times to determine the shortest time required to eliminate C. difficile from hospital rooms. So shorter cycle times may have been effective. Otter et al. (J Clin Microbiol 2009;47:205) found that C. difficile suspended in 0.3% BSA was eliminated in 30 minutes. Therefore, consider modifying the statement made in this paragraph.

   While exposure times to hydrogen peroxide vapor or mist may be as low as 30 minutes for significant reduction of C. difficile on surfaces, the systems generally have extensive setup and neutralization cycles that can occupy a room for hours. We modified the statement to encompass both "setup and disinfection" in a hospital room on line 231, page 12.

3. Page 17, Table 1. How could mean colony counts for C. difficile and VRE be less than 1 (0.23 and 0.47), when the lowest number of colonies listed in the ranges was 1 colony? It seems that the mean would have to be greater than 1. Also, if 28 sites were positive for C. difficile Before Sterilray treatment, and only 11 sites were positive After Sterilray treatment, then 17 sites must have revealed no growth after treatment. So wouldn’t colony count ranges for surfaces by 0-2 for C. difficile After Sterilray?

   Table 1 on page 17 shows the range of colony forming units that were recovered from positive surface cultures, however the mean was calculated from all of the surface culture results (positive and negative). We agree with the reviewer that this information is ambiguous, and for cohesion of the material presented the range should also encompass both positive and negative cultures. Therefore, the ranges were all changed to reflect positive and negative results (each range was changed to "0-x").
Also, how did you establish that some surfaces had MRSA colony counts of 1000? With most standard agar plates, it is nearly impossible to count more than 200 colonies on a single plate.

Plates that yielded more than 500 colony forming units were enumerated using Fotodyne's TotalLab Quant Analysis software (Fotodyne Inc., Hartland, Wisconsin). The software can be utilized to read plates with up to 6\log_{10} colony forming units. The manuscript was modified to describe colony counting procedure in the Methods section on lines 145-147 and 157-159. This limitation was also commented on in the Discussion section on lines 250-255.

Discretionary Revisions

1. Page 11, lines 226-227. The text states that the Sterilray was used “repeatedly”, without apparent adverse effects on surfaces. Was the device used more than 3 times on any given surface? If so, give some indication of how many times a single specific object was decontaminated, without effect.

For experiments conducted in hospital rooms of patients that had been discharged, items such as bedrails, call buttons and bedside tables were sampled. Depending on the flow of patients, the surfaces in each room were treated anywhere from 1 to more than 10 times over the course of this study. No apparent adverse effects were noticed on any of the surfaces that were treated more than 10 times. An additional note was added to line 234 in the Discussion section to define repeatedly as ">10 applications".

2. Page 12, lines 234-236. Consider mentioning that ATP assay systems could be used in future studies to establish the relative level of organic material on surfaces before use of the Sterilray device, and the impact on decontamination efficacy.

Before submitting this manuscript we discussed using ATP assay systems as an accessory measure to establish contamination on surfaces. However, using ATP assay systems may be misguiding for application of the Sterilray device. First, as indicated ATP assay systems detect ATP; the organic material that reduces the efficacy of the Sterilray device would not be detected using this assay unless there were concomitant bacterial contamination. Second, C. difficile spores do not produce ATP and would not be detected. One of the strong points for utilizing the Sterilray device is that it is effective on C. difficile spores, which are much more challenging to reduce than vegetative organisms. One could make a point that you may be more likely to find organic material and spores where you find ATP, but to our knowledge there have been no studies linking ATP with spores or organic material. We agree that the ATP assay systems can have impact on decontamination efficacy for many disinfectants, but ultimately feel that it may be disadvantageous for use with the Sterilray device. No changes were made to the manuscript.

In response to the reviewer Heike Martiny:

1. Discretionary revisions
   - throughout the text
   -- change mj to mJ, where applicable
The mj units were changed to mJ throughout the manuscript.

as dose is defined by time also, it would be correct to change to x mJ/cm² (~ y seconds)

The dose administered by the device is defined by time, distance of the device from the surface treated, and power setting (Methods section, "The Sterilray device"). If the device was held approximately the same distance from each surface you could achieve a specific radiant dose in approximately the same amount of time for each power setting (i.e. a radiant dose of 100 mJ/cm² in ~5 secs on the high power setting, line 163 page 8). For each experiment we described the power setting, approximate time of exposure and/or distance from the surface being treated, therefore no changes were made to the manuscript.

- headline line 195: please change to C. difficile Spores

"Spores" was left in lower case in the heading on line 95(?). No changes were made to the manuscript.

- Authors’ contributions: who is MJP?

MJP was erroneously placed in the authors' contributions section of the manuscript. Kevin Eckart (co-author) assisted in data collection for this study so the initials were corrected to KEE.

2. Major Compulsory Revision
Material and Methods - Reduction of pathogens by Sterilray device - line 136: Please explain how you validated the method to make sure, that only the spore form is left. As with organic load you showed the inhibition in reducing the microorganisms it it inexplicit why the device works in reducing spores within stool.

Here we used patient stool samples to simulate native C. difficile contamination. As stated on lines 137-138, samples were allowed to desiccate in room air for an hour to eliminate vegetative C.difficile. In our experience vegetative C. difficile does not survive in arid, oxygenated conditions for an hour. In a previous publication we found that vegetative C.difficile survived for up to an hour in room air on moist surfaces, however when allowed to desiccate in room air, vegetative C.difficile died in less than an hour (Jump et al. Antimicrob Agents Chemother. 2007;51:2883-7). This reference and elucidation of our previous findings was added to lines 139-142 of the manuscript to add validity to our method for eliminating vegetative C.difficile from the stool samples.

In response to the reviewer Scott Curry:
1. (major compulsory revision): The repeated use of "> 3-4 logs" and the like in describing the results of the log10 reductions in surface contamination is both colloquial and imprecise in the context of a disinfection manuscript. For almost all of the analyses in this report, there is a sufficient N such that mean and standard deviations of the relevant log10 reductions taken out to two significant digits should be reported. If the distribution of the log10 reductions is non-normal, the
medians should replace the means, but I presume from the use of the parametric
t-test in Table 1 that these log reductions have been formally tested for normality.
The CFU recovered for MRSA in Table 1 should be rounded to 28.2.

The manuscript was modified as suggested by reviewer. Log_{10} reductions were
reported out to two significant digits throughout the manuscript, with the exception of the
broad comparison of rapid log reductions of MRSA and VRE in comparison to C. difficile
spores (i.e. lines 37-39 and 193-195). The CFU recovered for MRSA was rounded to 28.2
in Table 1.

2. (major compulsory revision): In the methods section under the heading
"Preparation of C. difficile spores, MRSA, and VRE strains," the authors need to
clarify the medium used to enumerate C. difficile spores (and less essentially, for
VRE and MRSA) in the serial dilutions.

Our selective media for C. difficile, MRSA and VRE is described on lines 175-178
under the heading "Microbiology" in the Methods section of the manuscript. A line
referring the reader to this section for details on selective media was added to line 105
under the heading "Preparation of C. difficile spores, MRSA, and VRE strains".

3. (discretionary revision) In the methods section under the paragraph under
"Reduction of pathogens by the Sterilray device," a clarification of how the nine
20 mcl droplets (some containing extremely high cfu concentrations) were
collected from the Petri dish cover after UV irradiation would be helpful. Was this
again by swabbing or by flooding the cover with a known volume of diluent and
plating it? Were control experiments done to assess the efficiency of whatever
recovery method was devised?

A description of the method for recovering the droplets from the Petri dish cover
was added to lines 132-134 under the section "Reduction of pathogens by the Sterilray
device". The drops were collected by tipping the Petri dish cover and transferring the
suspenion into a tube. The suspensions were serially diluted and plated to enumerate
colony forming units. As described on lines 126-130 of this section, samples were left
untreated on the Petri dish cover (not irradiated) as a positive control. They were collected
in the same manner as the experimental (irradiated) samples. The positive control serves
as a baseline and also the standard for efficiency of our recovery method.

4. (discretionary revision) In the latter half of the methods paragraph under the
heading "Reduction of C. difficile on keyboards and portable medical equipment,"
clarification about the sampling process would help. For example, the authors
state that the inoculum is 10^4 - 10^5 spores in 10 mcl, but it is not clear how this
inoculum could be counted if picked up on a swab then plated directly to CDBA
unless it were first agitated in a known volume of diluent. Were the swabs also
inserted into their Amies medium carriers before plating in the lab, allowing
further dilution into that medium? There is a line here about "as described
previously," but there is no citation to further clarify this method or prior
experiements validating the efficiency of inoculum recovery.
In response to the statement "it is not clear how this inoculum could be counted if picked up on a swab then plated directly to CDBA unless it were first agitated in a known volume of diluent": as suggested by reviewer John Boyce (question 3), a description of the software for counting colonies on plates with greater than 500 CFU has been added to the manuscript.

It is unclear why the reviewer refers to "Amies medium carriers". The manuscript does not describe this type of carrier. Our collection swabs did not contain medium (BD BBL™ CultureSwab™, Becton Dickinson, Cockeysville, MD) so there is no concern for sample dilution. However, all samples were collected similarly. To assess reductions, untreated controls were compared to treated samples. By processing positive controls and experimental samples with the same collection technique, the method corrects for dilution effect on carriers, sensitivity of recovery, organisms remaining on carriers (unrecovered organisms), etc. This limitation is discussed on lines 250-255 of the Discussion. No change was made to the manuscript.

5. (discretionary revision) As hinted at above, I certainly agree with the limitations of the direct plating method that the authors point out in the last paragraph of the discussion section. Unless the lab personnel collecting the cultures were blinded to the pre- and post- Sterilray status of swab-sampled surfaces during both the collection and planting, however, there is the additional possibility of sampling bias in this study if more vigorous swabbing and/or plating of pre-disinfection surfaces occurred. There is also a serious implication of the authors' observation that the swab method has a sensitivity problem given the work of Trevor Lawley demonstrating that the infective dose for CDI in the mouse model is in the range of 1-5 spores/cm². Any method that does not result in complete disinfection of C. difficile from environmental surfaces may be inadequate. Because the issue of incomplete disinfection with existing hypochlorite and peroxide disinfectants is partly one of incomplete adherence to 10-minute contact times, the authors may want to comment more specifically on the future potential of UV-C devices to be used synergistically to accelerate the kill times of existing disinfectants. If future such studies are planned, it would be helpful to see side-by-side efficacy comparisons with existing disinfectants if the results are to be externally valid as effectiveness research.

In response to the reviewer's comment on sampling bias for collecting/plating samples with the swabbing method, we agree with the reviewer that not blinding the lab personnel is a limitation of this study. Native contamination is not homogenous, therefore we split each surface into half as described in the methods section and always swabbed the same half for the pre-disinfection and the other half as post-disinfection. Not varying our pre and post-disinfection sampling served two purposes: 1) to insure that pre/post samples were not accidentally switched by lab personnel, in our experience a simple study design allows for fewer processing errors, 2) to correct for the unascertained nature of contamination distribution on hospital room surfaces. We modified the paper to include these thoughts in the Discussion section of the manuscript, lines 255-259.

Regarding the reviewer's thoughts on the authors commenting "more specifically on the future potential of UV-C devices to be used synergistically to accelerate the kill times of existing disinfectants", on lines 225-227 we do suggest the Sterilray device as a potential adjunctive measure to other cleaning methods. One of the limitations of the Sterilray
device is that it only modestly reduced pathogens on surfaces that contained organic burden, so wiping down surface to reduce organic load may increase the efficacy of the device. However, we have no specific findings to support far-UV accelerating the kill times of existing disinfectants. It is unknown whether components of certain disinfectants have counteractive effects on far-UV just as found in the current study regarding organic load. Therefore, no changes were made to the manuscript.