Author's response to reviews

Title: Less and less - influence of volume on hand coverage and bactericidal efficacy in hand disinfection

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Author's response to reviews: see over
We would like to thank all reviewers for their helpful comments. They have certainly helped to improve the quality of our manuscript. Please find enclosed our point-by-point response:

**Referee 1 (Celso Cardoso)**

Discretionary Revisions

1. **Title, Page 1:** I suggest to replace “Lesser and lesser – the impact of volume on quality of hand coverage and antimicrobial efficacy in hand disinfection” by “Less and less – influence of volume on hand coverage and antibacterial efficacy in hand antisepsis”.

   Our comment: We have selected a new title which covers most of the suggested details.

2. **Abstract (pages 2-3) Background.** Line 1: replace “Some alcohol-based handrubs recommend using 1,1 mL per application.” by “The label on some alcohol-based hand rubs recommends using 1,1 mL of the product for effective disinfection of the hands.”

   Our comment: The wording has been changed to clarify the sentence.

3. Lines 5-7: the phrase - “All products were tested at 1.1 mL, 2 mL, and 2.4 mL; and 1 and 2 dispenser pushes; the foam product was tested at 1,1 mL, 2mL, and 2,4 mL foam.” - is not clear, please clarify.

   Our comment: The wording has been changed and is hopefully clearer now.

4. **Line 8:** “responsible application” should be explained in the abstract.

   Our comment: A brief explanation has been added.

5. **Conclusions.** Page 2-3, lines 3-5: In my opinion, the phrase “Infection-control practitioners should ensure patient safety by not reducing the volume of handrub recommended for adequate hand disinfection” should be removed. This statement (effectiveness) is not supported by the data presented (efficacy). This should also be considered in the conclusions (page10).

   Our comment: The sentence has been removed.

6. **Background (pages 4-5), Line 4:** “Rub hands until dry (IB)”. (IB) can be removed; otherwise, it should be explained to clarify to readers.

   Our comment: (IB) was removed.
7. Line 14: “(i.e., 60%-70% … v/v or w/w ? … ethanol).”
   Our comment: Unfortunately, this cannot be clarified because the cited study does not
mention this detail for the tested 10 gels. We know that some of the gels are labeled as v/v,
others as w/w. In this context, however, we have left the statement as it is.

8. Methods (pages 5-8), Handrub preparations. Line 4: rephrase: “(Purell Advanced Instant
Hand Sanitizer Foam; Gojo Industries).”
   Our comment: The wording was changed as proposed.

9. Product application. Line 7: the “responsible application technique” should be
   briefly described.
   Our comment: An explanation is now provided.

10. Assessment of untreated skin areas. Line 3: please include the source of
    “Dermalux Box” (i.e., company name, city, state and country).
   Our comment: The information has been added.

11. Efficacy according to ASTM E 1174-06. Line 1: rephrase: “ASTM E 2755-10”;
   Line 14: “Products were rubbed on the hands until dry”… What was the
   handrubbing technique used?; Line 15: Microbial samples were taken within 1
   minute after product application using sampling solution …”, How this was done?
   Line 18: rephrase: Butterfield’s phosphate buffer solution containing the same
   …”; Line 21: “A neutralizer assay was conducted according to ASTM E 1054-08”.
   This procedure should be briefly described. The ASTM E 1054-08 should be
   listed in the references section.
   Our comment: The hand rubbing technique is now described, the sampling technique is now
mentioned earlier, the sentence has been rephrased and is hopefully clearer now, the ASTM
1054 reference is now mentioned, and the neutralization validation procedure is briefly
explained.

12. Efficacy according to ASTM 2755-10. Rephrase: ASTM E 2755-10; in the
    subheading and line 1; Line 10: “Each product was rubbed on the hands until
    dry” What was the technique used?; Line 11: “Test products were effectively
    neutralized (data not shown)”. This procedure should be explained.
   Our comment: The method was rephrased. The technique is now described. The
neutralization technique is now briefly explained.
13. Results, Table 1. In the title, line 2, the clause “the foam product was applied in three additional foam volumes”; and the 7th column (1.1 ml foam), 8th column (2 ml foam), and 9th column (2.4 ml foam) can be removed (There are repeated data and empty cells). This information can be presented in the text. Our comment: The table legend was shortened, columns 7 – 9 removed, and the results presented in words in the result section.

14. Discussion, Page 9, line 9: rephrase: “Edmonds et al. (7) described …” Our comment: The wording has been changed.

15. Page 9, line 12: rephrase: “data in Edmonds et al (7) were …”. Our comment: The reference has been added.

16. Page 10, line 4: The observed incomplete coverage rates indicate that the WHO recommendation “cover all surfaces of the hands” is still fulfilled, but the technique we used seemed to be the best possible solution. Using other technique such as the six steps of EN 1500 or a volume < 2 mL are likely to jeopardize effectiveness goals. This text is not very clear as written, and should be reworded. Our comment: The paragraph has been reworded and is hopefully clearer now.

17. Conclusions, Page 10, the last phrase should be removed. Our comment: The sentence has been removed.

18. References, Reference 15: it should be completed. Our comment: The citation has been completed.

19. Reference 17: it is complete? Our comment: The citation has been completed.

Reviewer 2 (Jean-Yves Maillard)

Major compulsory revisions

1. The authors need to add more information on “responsible application” Our comment: A brief explanation is now provided in the abstract, some more information is provided in the method section (“product application”). We hope that the information now better allows to assessing how the products were applied in the study.
2. The authors need to provide a much better legend to the figures and to the scale applied. The reader needs to guess what colour is what and there seems to be some discrepancy between the level of coverage described in the text and between figure 1 and 2. The only difference between figure 1 and 4 (according to the legend) is 2 s contact time. I do not believe such a small difference in contact time has such a profound effect on hand coverage with AHRs. If the legend is correct then there is likely to be an issue with the reproducibility of the method, which will casts serious doubt as to the validity of the data.

Our comment: The legends have been completely reworded and are now hopefully self-explaining. The second part of the comment is also correct; it remains unclear why the three figures were chosen. The aim was not to compare different application times (as also proposed by reviewer 3, discretionary revision 2) but to show the coverage results for each of the three tested hand rubs with the corresponding recommended volume. That is why you see for both formulations recommended with 1.1 mL similar contact times with only 2 seconds difference. We have chosen this selection to make it clear how each hand rub exactly covers hands with the recommended volume. We consider this relevant for potential users. We have now explained the selection of figures in the results section and hope that the concern raised by the reviewer is adequately addressed.

3. The manuscript would benefit from a more detailed explanation of the analysis of the hand coverage by AHRs

Our comment: The whole paragraph has been rewritten and is hopefully clearer now.

4. Concerning the bacterial inoculated on hands, different volumes (no concentration mentioned) were used. How do these different volumes affect the results?

Our comment: That is correct, different volumes were used. The concentration of the bacterial cells per mL in the contamination fluid, however, is now mentioned (see also minor essential revision 3). Our data indicate that the contamination according to ASTM E 2755 with a lower amount of organic load (0.2 mL TSB) yields a slightly higher log reduction compared to the use of the contamination according to ASTM E 1174 with a higher amount of organic load on both hands (5 mL). It seems plausible to expect a better efficacy with a lower amount of organic load on hands because ethanol denatures protein in a non-specific mode of action. A paragraph has been added in the discussion to reflect this explanation.

5. Surprisingly, the authors do not want to make a correlation between smaller
volume and decrease in contact time on the hand. This is clearly indicated in Table 1 and I believe a decrease >10sec contact time will make a profound difference in activity. This is indeed highlighted in another study by Cheeseman et al. (2009), J Hosp Infect 2009, Vol.72(4), 319-325
Our comment: Yes, it may have been too obvious, many thanks for the comment. We have added a paragraph in the discussion section and included also the proposed reference.

Minor essential revisions

1. Page 6, first paragraph: it is unclear here and elsewhere in the manuscript (notably in Table 1) the volume dispensed by the dispensors. Here the authors use different volume and 1 or 2 dispenser pushes. There needs to be a more information provided even if the volume dispensed from the dispenser is not accurate.
Our comment: We have now explained the different types of dispensers (automatic and manual) and that we selected manual pump dispensers (“product application”). Using manual pump dispensers explains why the applied volumes do not correspond to the proposed amount per application. But this is likely to be real life in patient care.

2. Page 6, paragraph2, line 7: delete “on” after “with”: with standard hand drawing
Our comment: “on” was deleted.

3. Page 7, line 6: it is unclear why different volumes of Serratia marcescens were dispensed. This is not mentioned in the text. In addition the preparation of the bacterium and the concentration inoculated to the hand are not mentioned.
Our comment: The different volumes are required and described in the referenced ASTM test methods. As the experiments were exactly performed according to the referenced methods the volumes were exactly chosen as described. A brief statement and the references have been added. The preparation of the bacterium and the concentration of the bacterium in the contamination fluid are now described in both section (ASTM E 1174 and ASTM E 2755).

4. Page 9, paragraph 3, line 2: should read: “…as described in [4].”
Our comment: The wording has been changed.

5. Table 1: Note tested should be added to the blank cell (these can be merged)
Our comment: Based on comment 13 by reviewer 1 the data in columns 7, 8 and 9 were deleted and described with words in the results section. That is why there are no longer blank cells.

6. **Table 1: the volume dispensed by the dispensers should be mentioned.**
   Our comment: We are not quite sure about this comment. Whenever a fixed volume was applied it is mentioned (1.1 mL, 2 mL or 2.4 mL). For the pump dispenser pushes (one or two) we also have the mean applied volume in the table (column 3 in the categories “1 push per product” and “2 pushes per product”). Here you can see the volume dispensed per push. From our point of view the information is available. Please let us know if this comment is not satisfactory for you. May be the reviewer had something else in mind.

7. **Table 1: how many time the experiments was conducted. Add the number or repeats – the title mentions “Mean duration…”**
   Our comment: The number of subjects per experiments is now mentioned in the table legend.

8. **Table 2: add the number of repeats**
   Our comment: The number of subjects per experiments is now mentioned in the table legend.

9. **Table 3: the data coming from this study should be indicated**
   Our comment: They are now clearly indicated.

10. **The tables should not be a supplementary file.**
    Our comment: The tables are now part of the main manuscript.

**Reviewer 3 (Pengbo Liu)**

Major compulsory revisions

1. **For the antimicrobial tests, several variables (hand sanitizer types, ASTM versions, and neutralization) were introduced when the log reductions were compared between different volumes. These variables will make your results less conclusive and strong. In other words, while you want to determine the efficacy of different volumes, you need to diminish the confounding results from other variables.**
Our comment: We are not quite clear what exactly the respected reviewer wants us to review or to change, but we have tried an answer. If our answer is not appropriate, we kindly ask to try to explain again what is considered in this context as a major compulsory revision.

For the original data presented in the manuscript we have clarity on all variables such as the chosen hand rub, the ASTM version (the most recent one reflecting the current scientific standard) and the valid neutralization (also reflecting the current scientific standard). In that respect we consider our original data to be as sound as currently possible. And in that respect we consider our data also to be conclusive and strong. We know, however, that previously published data using the same formulation and using former versions of the same standard (ASTM E 1174) with maybe inappropriate neutralization may yield favorable results for the hand rubs which may well be explained by methodological differences to the current standard but do not necessarily demonstrate the efficacy on hands within the application time. We tried to explain this in Table 3.

2. The statistical methods were not powerful. For this type of data, two-way ANOVA, not chi-square, should be used to consider variances from both study groups (different volume groups) and subjects.

Our comment: We selected the chi-square test for a first statistical analysis because the endpoint of the study part on hand coverage is a frequency. As proposed by the reviewer we have now performed a two-way ANOVA and have changed the manuscript in the abstract, the method section, the results section. The overall result remained the same.

Discretionary revisions

1. The objectives of this study are clear but the scientific significance and practical application are my concerns. Unless handrub is covered all surfaces of the hands, do we really care about volume? There is another layer of question; surface area varies by people, some with large palm some with small. To set an optimal volume is not appropriate scientifically and practically.

Our comment: That is exactly the reason why we performed the study. If a manufacturer recommends a small volume such as 1.1 mL and considers it sufficiently effective it is likely to be used in clinical practice with such a small volume even if hands are rather large. That concern made us design the study. The practical implication should be to use a sufficient volume to cover both hands for the recommended application time. On large hands it will have to be a larger volume, as well as on dry hands. We realize that setting an optimal volume has limits and try to provide data that raise sound doubts on setting this optimal volume of 1.1 mL. And we hope that the reviewer can understand our motivation.
2. Figures 1, 2 and 3 provide confusing and less information. I could not see the differences of similar volumes of three products. Instead, I would like to see the images of different volumes with a specific product. For each figure, why the first two hands show the back of the hands and the next two are the palms?

Our comment: That is correct; we did not show the differences of similar volumes of the three products. We were able to show that the type of product has less influence on the frequency of completely covered hands by application of the two-way ANOVA. That is why we thought it is not so relevant to demonstrate this by figures. For clinical practice we thought that showing each product with its recommended volume might be the most interesting visual information for the readers and hope that the reviewer can understand that. We chose to show both the backs of the hands and the palms in order to allow each reader to see the entire hands.

3. Brief descriptions of ASTM and glove juice methods are necessary. Did you use both hands or only one hand? After hands were contaminated with S. marcescens, hand sanitizers were applied, and hands were rubbed one the other, were some of S. marcescens transferred to another hand?

Our comment: A brief description of ASTM has been added in the introduction. The glove juice method is now described once in the chapter “Efficacy according to ASTM E 1174-06”. Both hands were contaminated and both hands were used for hand antisepsis, that detail has been added in the method section. Hands were rubbed against each other (as in clinical practice) but since both hands were contaminated as briefly described in the method section it should not be regarded as a critical parameter.

Minor revisions

1. Need to follow up the guidelines of the MBC Infect Dis for making tables. Most journals do not allow vertical lines. Table title should be concise.

Our comment: The guidelines were reviewed and the tables changed accordingly.

2. Statistics part should be moved to the end of the Methods.

Our comment: The statistics part was moved to the end of the methods section.

3. Table 2. Why only the log reductions of small volumes were displayed? For each product, log reductions from all study volumes should be listed and compared.
Our comment: The study was designed to evaluate the efficacy of the three hand rubs with the volume recommended by the manufacturer (see also method section per efficacy test method). That is why we have not evaluated all three products with both volumes (1.1 mL and 2 mL) although it would have been interesting to do so.

**Additional comment by executive editor (Philippa Harris)**

*In addition please name the specific IRBs that approved the study within your methods section and clarify the country this research was carried out in.*

Our comment: The specific IRB and the country are now mentioned in both method sections.

**Additional change by authors**

Reference 15 (first manuscript version) has been published in the meantime with page numbers, the citation has been updated.

Blinding of investigators: We found a mistake in the first version of the manuscript. We wrote “Investigators were not blinded to treatment type”. This is not correct; the investigators were blinded to the type of treatment. This mistake has been corrected.