Peer Review File

Article Information: https://dx.doi.org/10.21037/ajo-21-40

We thank both reviewers for their valuable time taken to review our manuscript and for their insightful and important questions and points of clarification raised. We do apologise that with the changes made in manuscript, the latest line numbering has been altered (we have referenced new changes in altered line numbers)

Reviewer A:

Comment 1: Lines 198-204. **How was infectivity determined? Could the authors expand and explain how a lack of change in detectable RNA and, therefore, an absence of viral replication, translates to elimination of infectivity rather than a reduction or stagnation in infectivity?**

Reply 1: We apologise for the confusion. As outlined in the Design and Methods infectivity was determined by culturing the serially diluted virus post treatment in Vero cells for a 96-hour period. Growth was determined by a change in Ct score as measured by PCR of RNA extracted from these cultures between the inoculum (i.e., not cultured) and the 96 hour cultured samples. A lower Ct score in the cultured samples represents growth. A serial dilution was also used to "bracket" a range of dilutions expected to contain viable virus (based on the known titre of the inoculum) and higher dilutions in which virus was expected to be absent. Growth is either absent (*e.g.,* as represented in the samples that were not cultured or in the higher dilutions of the cultured samples which are too dilute to contain virus) or present. There is no concept of stagnation in infectivity since the virus readily grows in Vero cells if viable virus is present.

Changes in the text*:* We have amended the text starting at line 198-210 to better explain the experimental design.

Comment 2: Lines 208-210 **The authors should explain the rate of normal nasal mucociliary clearance in the results section of the in vitro study and also discuss the potential impact of an upper respiratory tract infection on nasal mucociliary clearance in the discussion.**

Reply 2: The normal rate of nasal mucociliary clearance has been estimated to be up to 20 minutes (Deborah S, Prathibha KM (2014) Measurement of Nasal Mucociliary Clearance. Clin Res Pulmonol 2(2): 1019). Many viruses, including common cold viruses such as rhinovirus and more serious pathogens such as SARS-CoV-2 have been reported to impair mucociliary clearance (Robinot, R., Hubert, M., de Melo, G.D. et al. SARS-CoV-2 infection induces the dedifferentiation of multiciliated cells and impairs mucociliary clearance. Nat Commun 12, 4354 (2021). https://doi.org/10.1038/s41467-021-24521-x)

Changes in the text*:* The text has been modified to include reference to the normal rate of mucociliary clearance at line 218-219. Similarly, the discussion has been amended at line 251-253 to include reference to mucociliary clearance in infected persons.

Comment 3: **The fact that a nasal spray is unlikely to reach the nasopharynx where the highest viral load and replication rate is found should also be mentioned in the discussion.**

Reply 3: The deposition of PVP-I in the nasopharynx is not yet known, but we acknowledge that it is likely to be lower than in the mid-turbinate and anterior nasal regions.

Changes in the text: A sentence has been inserted in the discussion at line 263-267

Reviewer B:

Comment 1: **Figure 1 – why show the lowest dilutions 1:6561 and 1:19683 in the 96 hours graph and not the 0-hour graph?**

Reply 1: The 1:6561 and 1:19683 dilutions were included to better "bracket" across the dilutions expected to contain infectious virus and dilutions at which the virus was no longer expected to be present (i.e., had been effectively diluted below detectable limits). This was only meaningful in the samples subjected to tissue culture expansion (i.e., the 96-hour sample)

Changes in the text: No change required

Comment 2: **"the control inoculum displayed robust replication of SARS-CoV-2, as indicated by the lower Ct scores (higher viral RNA copies) throughout the dilution series" But in the graph this is not evident at the higher dilutions! There is no visible change from dilutions 1:243 onwards**

Reply 2: This is as expected. The dilution range was chosen to "bracket" across dilutions expected to contain infectious virus and dilutions at which the virus was no longer expected to be present (i.e had been effectively diluted below detectable limits). At dilutions of 1:243 and above the virus was evidently diluted below detectable limits as expected

Changes in the text: No change required

Comment 3: "**Given the well documented difficulty of culturing virus at Ct values above 25, it is not surprising that culturable virus could not be isolated from these samples" - In Table 2 it appears that ALL your subjects had Ct values over 25 ? does this show an issue with the transport of specimens ?**

Reply 3: It is difficult to absolutely rule out a loss of infectivity between collection and assay, which is true for all such studies. We have no evidence of a loss of cold chain and samples were collected and stored in appropriate Universal Transport Media. However, the Ct scores above 25 are not necessarily diagnostic of a transport issue. Ct scores in this range are common and in the instance in which viable virus could not be recovered, likely represent subjects that may have ceased shedding but have yet to clear residual RNA. The issue we believe is the well documented difficulty in isolating viable virus from samples with high Ct scores, but of course it is not possible to rule out other possibilities

Changes in the text: No change required

Comment 4: **Subjects 5 and 21 had variable responses and appeared to have quite high titres even at 60mins how does this translate with infectability ie would they still be infective?**

Reply 4: The titres recorded are actually low in all cases, at only 2 log units. Notably, van Kampen *et al* report that viral loads above 7 log10 RNA copies/mL are more commonly associated with isolation of infectious SARS-CoV-2 from the respiratory tract and the probability of isolating infectious virus was less than 5% when viral RNA load was below 6.63 Log10 RNA copies/mL (van Kampen, J.J.A., van de Vijver, D.A.M.C., Fraaij, P.L.A. et al. Duration and key determinants of infectious virus shedding in hospitalized patients with coronavirus disease-2019 (COVID-19). Nat Commun 12, 267 (2021). https://doi.org/10.1038/s41467-020-20568-4). Despite these observations, we can only speculate, but the fact that infectious virus was isolated suggests that the subjects could be a transmission risk, albeit at a low level

Changes in the text: No change required

Comment 5: **Figure 2 – how are the confidence interval extending below 0% at 60minutes if all subjects "at 60 minutes post-dose, virus titers were below the respective baseline values in all 6 subjects"**

Reply 5: The intervals shown are standard deviations around the mean which in variable data sets can be larger than the mean. Although we appreciate that practically it may have made sense to truncate the deviation at 0%, we chose not to do so for transparency reasons

Changes in the text: No change required

Comment 6: Assuming the 5 and 15 minute CI represent the increase in subject 21"In the in vivo study, the fact that 8 out of 14 confirmed COVID-positive samples did not yield viable virus for cell culture may reflect just how sensitive the PCR test is" - BUT given these were "laboratory-confirmed (PCR), COVID-19 positive subjects with recent COVID-19 symptoms (within 5 days of onset)." Does this not again bring into question the transport and logistic issues rather than false positive PCR tests

Reply 6: Please refer to answers to comments 3 and 4. Isolation of viable virus from laboratory confirmed PCR samples with Ct above 25 is reported to be difficult. We do not believe the PCR results to be false positives. In fact, the South African PCR data were independently confirmed with a second PCR assay conducted at PathWest in Australia.

Changes in the text: No change required

Comment 7: **In limitations you should mention the generalisability of these results to other COVID variants. Line 283 COVIS-19 - change to COVID -19**

Reply 7: Thank you for the observation

Changes in the text: We have amended the text as suggested in line 286-291