








BRIEF REPORT

REVISED Effect of elevated temperature on SARS-CoV-2 viability

[version 2; peer review: 2 approved, 1 not approved]

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a worldwide disruption of global health putting healthcare workers at high risk. To reduce the transmission of SARS-CoV-2, in particular during treating the patients, our team aims to develop an optimized isolation chamber. The present study was conducted to evaluate the role of temperature elevation against SARS-CoV-2 viability, where the information would be used to build the isolation chamber. 0.6 mL of the Indonesian isolate of SARS-CoV-2 strain 20201012747 (approximately 10^{13} PFU/mL) was incubated for one hour with a variation of temperatures: 25, 30, 35, 40, 45, 50, 55, 60, and 65°C in digital block heater as well as at room temperature (21-23°C) before used to infect Vero E6 cells. The viability was determined using a plaque assay. Our data found a significant reduction of the viral viability from 10^{13} PFU/mL to 10^9 PFU/mL after the room temperature was increase to 40°C. Further elevation revealed that 55°C and above resulted in the total elimination of the viral viability. Increasing the temperature 40°C to reduce the SARS-CoV-2 survival could create mild hyperthermia conditions in a patient which could act as a thermotherapy. In addition, according to our findings, thermal sterilization of the vacant isolation chamber could be conducted by increasing the temperature to 55°C. In conclusion, elevating the temperature of the isolation chamber could be one of the main

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Approval Status   

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version 1 08 Apr 2022	 view	  view	

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variables for developing an optimized isolation chamber for COVID-19 patients.

Keywords

COVID-19, Isolation chamber, SARS-CoV-2, Temperature, Transmission

Any reports and responses or comments on the article can be found at the end of the article.



This article is included in the **Emerging Diseases and Outbreaks** gateway.

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Author roles: **Harapan H:** Conceptualization, Formal Analysis, Methodology, Project Administration, Resources, Supervision, Visualization; **Johar E:** Data Curation, Formal Analysis, Resources; **Maroef CN:** Data Curation, Formal Analysis, Methodology, Resources; **Sriyani IY:** Data Curation, Formal Analysis, Methodology, Resources; **Iqhrammullah M:** Writing – Original Draft Preparation, Writing – Review & Editing; **Kusuma HI:** Writing – Original Draft Preparation, Writing – Review & Editing; **Syukri M:** Conceptualization, Supervision; **Razali R:** Conceptualization, Supervision; **Hamdani H:** Conceptualization, Supervision; **Kurniawan R:** Conceptualization, Supervision; **Irwansyah I:** Conceptualization, Supervision; **Sofyan SE:** Conceptualization, Supervision; **Myint KS:** Data Curation, Formal Analysis, Methodology, Resources; **Mahlia TMI:** Conceptualization, Supervision; **Rizal S:** Conceptualization, Funding Acquisition, Supervision

Competing interests: No competing interests were disclosed.

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REVISED Amendments from Version 1

In this new version, we have added the information that this research is useful for people living in the tropics in the Introduction section. We have added more important information in the Methods section such as: (a) the description for virus propagation; and (b) information related to the SARS-CoV-2 used in this study such as the origin and the lineage. In Discussion section we have removed the hypothesis that lifting the ambient temperature around an infected patient for one hour to 40°C might reduce the risk of infection to health care workers. We have added more limitations of our study including: (a) we did not assess the role of other factors that might influence the SARS-CoV-2 transmission such as humidity; and (b) the sensitivity of the virus to temperature was tested in a liquid state only and we did not assess the aerosol state.

Any further responses from the reviewers can be found at the end of the article

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has inflicted disruptions in many aspects of health systems globally. SARS-CoV-2 is an enveloped, non-segmented, positive sense, single-stranded RNA virus with a genome of approximately 30 kilobases.^{1,2} The virus is mainly transmitted by nasopharyngeal droplets of an infected person; however, it could also be transmitted through aerosol.³ The viability of the virus in the environment is influenced by several factors, including climatic parameters such as humidity and temperature.⁴ Temperature has been proposed as a factor that affects SARS-CoV-2 transmission.⁵ Previous studies found that the temperature effected the transmission of the SARS-CoV-2 in which high environmental temperature reduced the number of COVID-19 cases.^{6–8} A study in State of Rio de Janeiro, Brazil found that the maximum and average of temperature were correlated negatively with COVID-19 infection.⁶ Data from 117 countries also found a negative association between temperature and COVID-19 transmissibility in which an increase of 1°C could decrease COVID-19 prevalence by approximately 5.4%.⁷ Several other investigations, however, have shown no evidence of a substantial influence of temperature on SARS-CoV-2 transmission.^{9,10}

Nevertheless data reveals that SARS-CoV-2 is highly susceptible to heat.¹¹ A recent study has shown that the virus could survive for at least 14 days at 4°C while only two days at 37°C and five minutes at 70°C.¹¹ Another study suggested that, at 40°C SARS-CoV-2-infected epithelial cells have reduced viral transcription and replication.¹² Although studies on the effect of elevated temperature on SARS-CoV-2 have been carried out, the temperature ranges used are limited.¹³ In addition, the effect of temperature on viruses originating from Indonesia has not yet been published. As part of our project to optimize the isolation chamber for COVID-19 patients, we determined the effect of temperature on the resistance of SARS-CoV-2 originating from Indonesia by evaluating the viral viability with a range of temperatures from room temperature (21–23°C) to 65°C. Understanding viral survivability is critical for developing a temperature optimized isolation chamber that could minimize the risk of infection to healthcare workers and optimize energy consumption while ensuring comfort for patients. In addition, the information of this study might important for those who are living in the tropics.

Methods**SARS-CoV-2**

SARS-CoV-2 strain 20201012747 isolated from Jakarta, Indonesia was used in this study. The virus originated from a patient with severe COVID-19 manifestation. The virus was kindly supplied by the Eijkman Institute for Molecular Biology. Before used in this study, the virus has been passaged twice on Vero E6 cells (ECACC, Vero C1008) (RRID: CVCL_0574). The virus was classified as an ancestral SARS-CoV-2 strain and isolated on 12 October 2020.

Vero E6 cells

The Vero E6 cells were maintained in Modified Eagle Media (MEM) (Cat. no. 11090081) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Cat. no. 26140095), 3.5 mM Na₂CO₃ (Cat. no. 25080094), 1% penicillin-streptomycin-amphotericin B (Cat. no. 15240062), 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Cat. no. 15630080), 1% non-essential amino acid (Cat. no. 11140050), and 2 mM L-glutamine (Cat. no. 25030081). All were from Gibco (Thermo Fisher Scientific, MA, USA). The cells were seeded into 12-well clear bottom plates (Cat. no. 3513, Corning, USA) for plaque assay.

Exposure of temperatures

To expose to different temperatures, 0.6 mL of SARS-CoV-2 stock in 1.5 mL sterile tubes were incubated at room temperature RT (21–23°C), 25, 30, 35, 40, 45, 50, 55, 60, or 65°C for 1 hour in a digital block heater (Cat. no. 5382000031, Eppendorf, Germany). A separated experiment was conducted for each temperature; three replicates were used for each temperature and each replicate was repeated three times.

Plaque assay

After the incubation at different temperatures, the viruses were diluted with a 10-fold serial dilution with 2% MEM (10^{-1} to 10^{-12}) and 0.1 mL was inoculated onto 95-100% confluent monolayers of Vero E6 cells for 1 hour at 37°C with 5% CO₂ with manual gentle shaking every 20 mins. After 1 hour of incubation, each well was then covered with 1 mL of 2% carboxymethyl cellulose (Cat. no. 17854-1KG, Merck, Germany) containing MEM, 2% FBS (Cat. no. 26140095), 3.5 mM Na₂CO₃ (Cat. no. 25080094), 25 mM HEPES (Cat. no. DMEM 15630080), 1% non-essential amino acid (Cat. no. 11140050), and 1% penicillin-streptomycin-amphotericin B (Cat. no. 15240062); All from Gibco (Thermo Fisher Scientific, MA, USA). The plates were incubated in cell incubator for 72 hours at 37°C and 5% CO₂. The cells were then fixed with 4% paraformaldehyde for 4 hours at room temperature, and stained with 1% crystal violet. The plaques were counted manually. All works with infectious SARS-CoV-2 were conducted in the Biosafety Level 3 Laboratory at the Eijkman Institute for Molecular Biology in Jakarta. The plaque forming unit (PFU/mL) was calculated by dividing the number of plaques by dilution factor.

Results

The virus was observed to remain stable from RT to 35°C with an average plaque count of 10^{13} PFU/mL.²³ A reduction in the viral count was observed in the 40°C treatment group at 10^9 PFU/mL. Increasing the temperature to 45°C resulted in a further reduction of the viral viability resulting in 10^6 PFU/mL. The last temperature with visible plaque was in the 50°C treatment group with a result of 10^2 PFU/mL. The reduction of SARS-CoV-2 viability had a temperature-dependent trend within the temperature range of 35 to 50°C (Figure 1). No plaques were visible in the 55, 60 and 65°C treatment groups.

Discussion

Nosocomial transmission of SARS-CoV-2 has been identified to occur via multiple routes in healthcare facilities¹⁴ indicating that uncomplicated measures like wearing personal protective equipment along with surface cleaning and decontamination¹⁵ could be used effectively to reduce the transmission of infection. Other than that, modification to facilities such as, including isolation chambers with temperature control could help minimize the transmission.^{11,16} As noted in a previous study,¹¹ temperature affects the stability of the virus in aerosol or on a surface. When the temperature of the room is not elevated, SARS-CoV-2 could remain stable for up to 72 hours on a surface such as plastic and stainless steel and three hours in aerosols.¹⁶ Based on the findings of this present study, to reduce the viral viability significantly, a higher room temperature might be important. However, according to a previous report, the average maximum

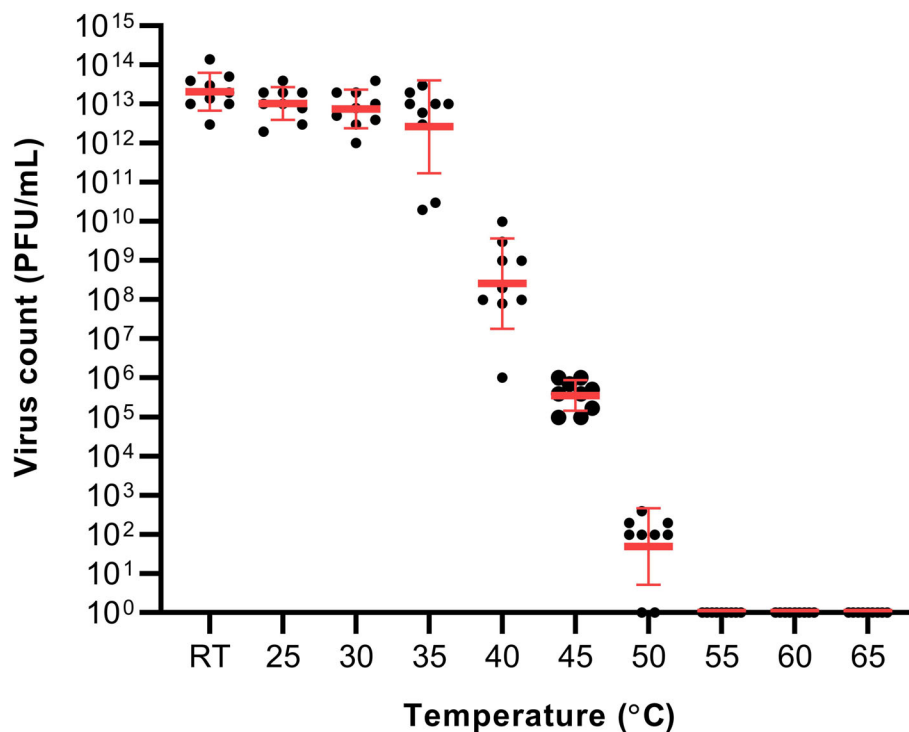


Figure 1. The effect of temperature on severe acute respiratory syndrome coronavirus 2 viability. RT - (21-23°C).

temperature for Indonesian people to still feel comfortable falls between 24 and 29°C¹⁷ and to increase the room temperature would probably cause discomfort to the patient.

The administration of heat to the isolation chamber could be conducted prior to patient handling to reduce the likelihood of SARS-CoV-2 transmission. The other possibility is using heat in the isolation chamber as a means of thermotherapy. Thermotherapy is where mild-temperature elevation or hyperthermia (39–42°C) is used as a treatment against SARS-CoV-2 infection.¹⁸ Previous studies assessing the indoor temperature and SARS-CoV-2 were associated inactivation of SARS-CoV-2.^{19–21} Following the increase in temperature, heat-shock proteins (HSPs) are released which downregulates the progression of sepsis-induced acute lung injury.²² However, HSPs could become hosts to several viruses (such as human papillomavirus, adenovirus, and dengue virus) promoting their infectivity.²³ In the case of SARS-CoV-2, its infectivity is more likely to be degraded than promoted by the HSPs.¹⁸ Therefore, heat administration to the isolation chamber should not be performed on COVID-19 patients with human papillomavirus, adenovirus, or dengue virus co-infections. In addition, it should not be attempted on patients with severe-to-critical COVID-19 as they would be more likely to have an increased risk of mortality following the thermotherapy or heat administration.²⁴

One of the limitations of this study was we did not assess the role of other factors that might influence the SARS-CoV-2 transmission such as humidity. Therefore, the results of our study should be incorporated with data of the other studies assessing the effects of humidity on SARS-CoV-2 viability. In addition, we assessed the effect of the temperature on SARS-CoV-2 in a liquid state only and this might have influenced the results. Assessing the effect of the temperature on different states might could provide better understanding.

Conclusions

This present study also has proven that increasing indoor temperature to 55°C is sufficient to terminate the virus. Further increment to the temperature would not be necessary and only results in higher energy consumption. Similarly, a previous study also reported the inactivation of 90% of SARS-CoV-2 achieved at 54.5°C after 36 minutes.¹³ However, for use in treatment 55°C, might be too high for patients to tolerate. In that case, we only suggest the use of such temperature to thermally sterilize the isolation chamber prior to its use.

Data availability

Underlying data

Figshare: Effect of elevated temperature on SARS-CoV-2 viability. DOI: <https://doi.org/10.6084/m9.figshare.19243515.v1>.²⁵

This project contains the following underlying data:

- Master Table.xlsx [Table containing the raw data of the study]

Data are available under the terms of the [Creative Commons Attribution 4.0 International license \(CC-BY 4.0\)](#).

Ethics statement

Not applicable.

Acknowledgement

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Current Peer Review Status:   

Version 2

Reviewer Report 22 September 2023

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Katherina Sewald 

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The authors addressed my questions and included limitations of their study into the discussion.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Expertise in in vitro / ex vivo infection research, focus on respiratory diseases.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 24 August 2023

<https://doi.org/10.5256/f1000research.144182.r193572>

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This study involves a single experiment to demonstrate the effect of elevated temperature on SARS-CoV-2 stability. The experimental setting and findings were very similar as the previously published report. One of the major purposes of the authors is to apply the findings to develop a temperature optimized isolation chamber in a hospital setting. However, I have reservation on the conclusion that elevating the temperature of an isolation chamber with COVID-19 patient will

reduce the risk of transmission to the healthcare worker.

On one hand, patient continuously shed virus to the environment. Provided that respiratory droplets and aerosols are the major routes of transmission, pre-heating the chamber for one hour before the healthcare worker coming in will have minimal effect on the continuous droplet and aerosol generation. If the purpose is to reduce the risk of fomite transmission, simple surface disinfection will be more efficient and safer. The authors provided evidence by other groups that report negative association between air temperature and COVID-19 transmission in different countries. However, these findings are controversial. Many other studies found no evidence on higher temperature will have negative impact on COVID-19 transmission and some even found the reverse.

On the other hand, it may not be desirable to place a patient in an undesirable environment at 40 degree C, not to mention the increased risk of heat related illness, like heat exhaust or heat stroke, which can be lethal. I think this needs to be considered very carefully. In addition, it is mentioned in the abstract that increasing the environmental temperature to 40 degree C will create mild hyperthermia condition in patient and reduce SARS-CoV-2 survival. However, there is no evidence to support this claim. The viability of virus in culture medium without present of any cell may not be the same as in the respiratory track covered with epithelial cells at the same temperature.

There are other flaws in this manuscript including the experimental design and data presentation. The viability of the virus was only studied in medium. Although the authors mentioned this as a limitation of the study, assessing of the viral stability in droplet / aerosol exposed to hot air would be more relevant and essential for coming to the current conclusion. Plaque assay is fine for determining the viral titre of infectious virus. However, the information on limit of detection of the assay is missing. No plaque observed in the assay does not necessarily mean that the viral titre is zero. The input control without one hour incubation was also missing, so one has no idea about the initial titre. Statistical analysis was also missing.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Virology

We confirm that we have read this submission and believe that we have an appropriate level of expertise to state that we do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Version 1

Reviewer Report 30 June 2022

<https://doi.org/10.5256/f1000research.121896.r137526>

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The authors addressed the research question whether elevated temperature has an effect on SARS-CoV-2 infectivity. The question might be important to establish conditions in e.g. isolation chamber which reduces the transmission of SARS-CoV-2. There are already data about the temperature sensitivity of SARS-CoV-2 available, suggesting that SARS-CoV-2 is susceptible to temperature changes, in particular to heat. This temperature sensitivity influences the virus' ability to replicate. Systematic approaches to assess the temperature sensitivity in detail is so far missing. This gap was filled by the herein submitted work. In general, the manuscript is well written, easy to understand.

The methods were adequate to answer the research question. Choosing the plaque assay for determination of infective virus particles is sufficient. Using PCR would add no further information. Any description for virus propagation is missing. As the process and purity might have an influence on the results, I would add that information. Temperature steps were chosen in 5 °C intervals. I wondered why more physiological temperature were not also chosen, such as 31°C, 34°C...(as representatives in tracheal temperature when breathing e.g. 20°C warm air). This was recently addressed by <https://doi.org/10.1093/infdis/jiac264>. The sensitivity of the virus to temperature was only tested in a liquid state. The question about the stability as virus aerosol remained unanswered. It is a pity that the authors did not compare different virus strains. They focused only on SARS-CoV-2 strain 20201012747 isolated from Jakarta, Indonesia. I found no information about the Statistics used. I also missed some recent literature found by literature search.

In general, I was not really convinced by the hypothesis that lifting the ambient temperature

around an infected patient for one hour to 40 degree would substantially reduce the risk of infection to health care workers. As infection progresses, any exhalation of patient' breath would increase the virus load in the air again. It is also necessary to understand what breathing of 40 °C warm air means for the air temperature in the trachea and in the alveoli as it could be reduced to core body temperature again – meaning that it remains without any effect if we think of using that for treatment of patients. Other methods for lifting body temperature might be more effective.

It is hard to say whether the information has great importance for others as the information provided is not substantially new. Other researchers might be interested in reading the study, in particular researchers focusing on transmission of SARS-CoV-2. Overall, this is a very small publication showing little results. These results are not outstanding, in terms of the content of the results per se and not in terms of the methods/techniques used and also not in terms of the effort involved, which might also justify indexing.

Minor point:

- M&M part: please change from two times to twice

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Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Expertise in in vitro / ex vivo infection research, focus on respiratory diseases.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have

significant reservations, as outlined above.

Reviewer Report 13 April 2022

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Methods of preventing the spread of Covid-19 are continuously being studied, environmentally friendly methods have a positive impact on the community. The research is very good and useful for health workers and the public, so it deserves to be indexed. In the future, this research will be a reference for policy makers in dealing with COVID-19.

Introduction

- Add that this research is useful for people living in the tropics.

Discussion

- Temperature is not the sole cause of disease breeding, so in this section it is necessary to add research limitations, such as humidity factor which are not included in this research study.
- It is advisable to add research related to indoor temperature.

Conclusions

- "This present study also has proven that increasing temperature" -> Indoor temperature

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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