

Thermal Inactivation of Aerosolized SARS-CoV-2

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide with its different variants. The transmission efficiency of the new variants is much higher than the existing ones. Therefore, developing new preventive measures based on the transmission routes of the virus is needed to limit the spread. The possible transmission routes include direct contact with surfaces contaminated with droplets secreted by patients and airborne viral transmission from person to person. Thermal inactivation is a preventive measure that applies high temperature to objects or fluids, as has been reported previously. However, inactivation data of aerosolized SARS-CoV-2 exposed to heat for a short time at high temperatures are not in the literature yet. We evaluated the inactivation of the aerosolized virus while passing through an electric heater. The virus inactivation test experiments were conducted at two temperatures of the heater's outlet air, 150 ± 5 oC, and 220 ± 5 oC, at an air flow rate of 0.6 m³/h (10 L/min) and heat exposure time of 1.44 s. The loss in viability of the virus at 150 oC and 220 oC was measured as 99.900% and 99.999%, respectively. The results indicate that the high-temperature inactivation of SARS-CoV-2 may potentially reduce aerosolized viral indoors.

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide with its different variants. The transmission efficiency of the new variants is much higher than the existing ones. Therefore, developing new preventive measures based on the transmission routes of the virus is needed to limit the spread. The possible transmission routes include direct contact with surfaces contaminated with droplets secreted by patients and airborne viral transmission from person to person. Thermal inactivation is a preventive measure that applies high temperature to objects or fluids, as has been reported previously. However, inactivation data of aerosolized SARS-CoV-2 exposed to heat for a short time at high temperatures are not in the literature yet. We evaluated the inactivation of the aerosolized virus while passing through an electric heater. The virus inactivation test experiments were conducted at two temperatures of the heater's outlet air, 150±5°C, and 220±5°C, at an air flow rate of 0.6 m³/h (10 L/min) and heat exposure time of 1.44 s. The loss in viability of the virus at 150°C and 220°C was measured as 99.900% and 99.999%, respectively. The results indicate that the high-temperature inactivation of SARS-CoV-2 may potentially reduce aerosolized viral indoors.

Keywords: SARS CoV-2, Aerosolized Coronavirus, Covid 19, Thermal inactivation, High Temperature

1 | INTRODUCTION

The new variance of SARS-CoV-2 has a 43% to 90% higher reproduction number than the existing variants (Davies et al., 2021). Possible transmission routes include airborne particles, respiratory droplets, and contact with a contaminated surface. Interrupting the chain of the transmission routes is vital to limit the spread of the virus. One of the inactivation methods is high-temperature exposure of contaminated surfaces or liquids, which has been reported previously (Darnell et al., 2004; Hessling et al., 2020; Liu, 2004). Heat inactivation of SARS-CoV-2 has mainly been used to sterilize contaminated personal protective equipment such as masks and gloves in hospitals and contaminated equipment and liquids in laboratories before reuse (Abraham et al., 2020; Batejat et al., 2021; Biryukov et al., 2021).

SARS-CoV in liquid was inactivated in 45 minutes and 75 minutes at a temperature of 75 °C and 56 °C, respectively (Darnell et al., 2004). A 4 log₁₀ TCID₅₀ reduction was observed with a heat treatment protocol of 60°C - 60 min and 92 °C - 15 min (Pastorino et al., 2020). Using the SARS-CoV and SARS-CoV-2 data from different studies, the time required for 5 log₁₀-reduction was estimated as 32.5, 3.7, and 0.5 minutes for temperatures of 60 °C, 80°C, and 100 °C (Hessling et al., 2020). A 6 log₁₀ TCID₅₀ reduction was obtained within a fluidic system within 1.03 s at a temperature of 83.4 °C (Jiang et al., 2021). Nevertheless, dry heat inactivation of aerosolized SARS-CoV-2 has not been investigated yet.

During the Covid-19 pandemic, exposure to indoor aerosolized SARS CoV-2 has become one of the primary challenges (Chia et al., 2020; Morawska et al., 2020). Heat inactivation of the aerosolized SARS-CoV-2 is one way to reduce the spread of 2019 coronavirus disease (COVID-19). In the present study, the inactivation of SARS-CoV-2 at high air temperatures of 150 °C and 220 °C has been investigated using an experimental setup, while minimizing potential hazards.

2 | MATERIALS AND METHODS

Preparation of SARS-CoV-2 Suspensions Experiments were performed in biosafety level 3 (BSL3) facilities, using a stock suspension of SARS-CoV-2 strain (Gen Bank No: MT955161.1). SARS-CoV-2 virus stock was prepared by inoculating the Vero E6 cell line in Dulbecco's modified Eagle's medium (DMEM-10). Dulbecco's modified Eagle's medium containing supplements (10% fetal bovine serum, 2mM/ml L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, and 0.5 mg/ml fungizone (Amphotericin B)) was added to the flask, and the cells were incubated at 37°C for 72 h. The supernatant was collected, clarified by centrifugation, and stored at -80°C. TCID₅₀ titer was determined by the Spearman- Karber method as described (Hubert, 1984).

Experimental Setup for Heat Inactivation of SARS-CoV-2 The experimental system is shown in Fig.1, consisted of an air compressor with an air flow rate of 0.6 m³/h (10 L/min), an electric heater (600 Watt), a venturi injector, an air flow meter (RST Measurement Control Tech., Istanbul, Turkey), a nebulizer (M102,

Jiangsu Yuyue Medical Equipment & Supply Co., Ltd., Danyang Jiangsu, China) with a nebulization rate of 0.2 mL/min, with an average particle size of 3.7 microns, an inline polycarbonate filter holder with a gelatin membrane filter (Sartorius, Gottingen, Germany), a thermometer with k-type thermocouple (CEM-613, CEM, Shenzhen, China), and an aspirator (Ecoaspir, Kare Medical, and Analytical Devices Ltd. Co., Ankara, Turkey) for vacuuming. Suspension of the SARS-CoV-2 was nebulized into the venturi injector and mixed with the compressor's air before entering the electric heater. The temperature of the air increased through the heater. Since the gelatin filter's maximum working temperature was 60°C, the silicone house connected the heater's outlet and the filter holder wound around the ice to reduce the temperature below 60 °C. The aspirator with an airflow of 0.6 m³/h was connected to the filter holder output and used as a vacuum pump.

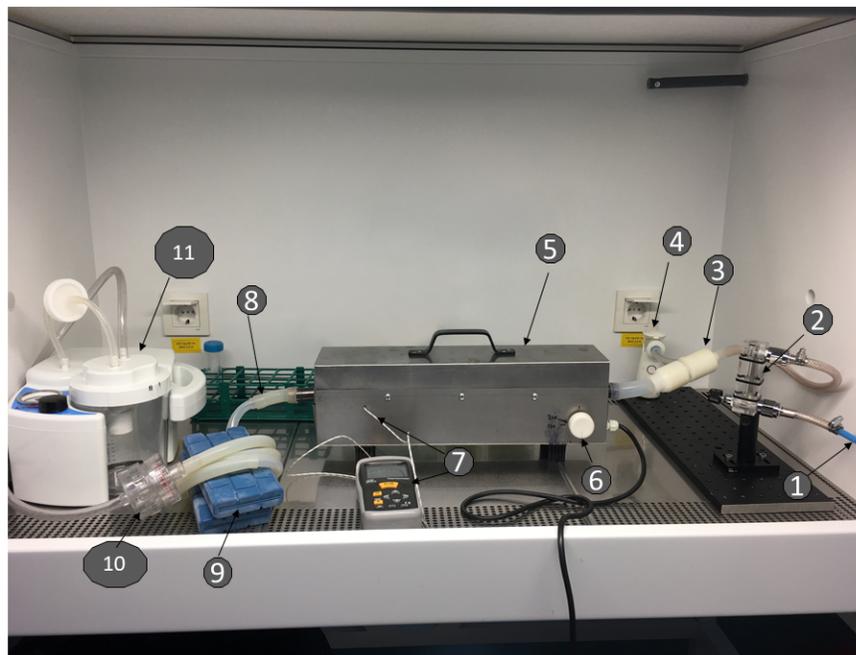


FIGURE 1 1) Hose between the air compressor and air flow meter, 2) Air flow meter, 3) Venturi injector, 4) Nebulizer, 5) Heater, 6) Heater control knob, 7) Thermometer with K-type thermocouple, 8) silicone hose, 9) Ice molds, 10) Gelatin filter holder, 11) Aspirator

A detailed illustration of the electric heater is shown schematically in Fig. 2. The body of the heater was made of a metal sheet with a thickness of one mm. In the heater, an electric heating coil was located perpendicular to the air flow and used as a thermal energy source. The heater's airway cross-section area is 4x4 cm² with a length of 25 cm. The heating coil occupies 15 cm of the heater. The thermocouple was located at the outlet of the heater to measure the outlet temperature. Outside of the heater was isolated using a fiberglass slab.

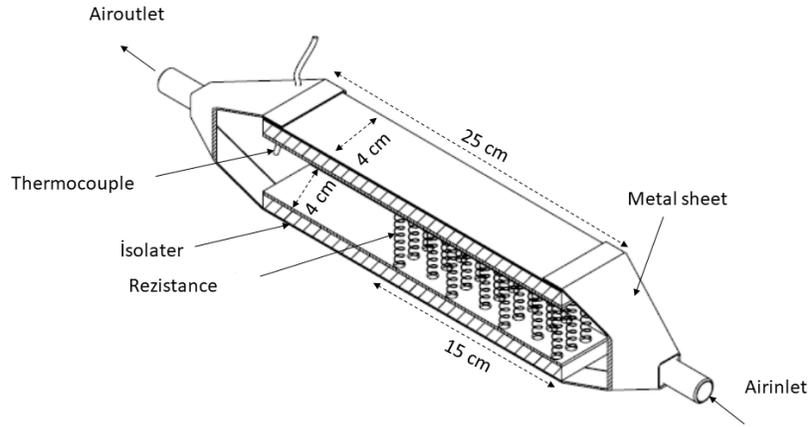


FIGURE 2 Detailed schematic presentation of the heater.

In the control experiment, the heater was off, the compressor and the nebulizer were on for five minutes. The control experiments were repeated twice. In the test experiments, the heater’s outlet temperature was set to $150\pm 5^{\circ}\text{C}$ and $220\pm 5^{\circ}\text{C}$. This was accomplished by varying the current through the electric heating coil. The air flow rate was set to $0.6\text{ m}^3/\text{h}$ in all the experiments. In the test experiments, the compressor and the heater were turned on, and then the outlet air temperature was set to one of the above temperatures. Then, the nebulizer was turned on. After five minutes, the nebulizer was turned off, then the heater and the compressor were turned off. After the experiments, the gelatin filter inside the inline polycarbonate filter holder was dissolved in distilled water to harvest the virus.

3 | RESULTS AND DISCUSSION

The viral presence of the stock virus was $7.5\text{ log}_{10}\text{TCID}_{50}$. The control experiments were performed twice, and the average viral load was $5.5\text{ log}_{10}\text{TCID}_{50}$. In setting the heater outlet air temperature to $150\pm 5^{\circ}\text{C}$, the viral load was reduced to $2.5\text{ log}_{10}\text{TCID}_{50}$. The reduction in the viability of the virus is 3 log_{10} or 99.9%. At the higher temperature of $220\pm 5^{\circ}\text{C}$, the virus load viability was $0.5\text{ log}_{10}\text{TCID}_{50}$, reducing the infectivity of the virus to 5 log_{10} or 99.999%.

Table 1 $\text{log}_{10}\text{TCID}_{50}$ values of the stock virus, control, and test groups.

TEST	Experiments	($\text{Log}_{10}\text{TCID}_{50}$)	Mean	The average reduction in the viral load	The average reduction in the viral load
Virus Titration	Stock virus	7.5	-	$R = \log_{10} C - \log_{10} T$ $R = 3.00$	%99.900
Virucidal Test (5 min at 150 °C)	Positive Control 1	5.00	5.50		
	Positive Control 2	6.00			
	Test 1	2.5	-		
Virucidal Test (5 min at 220 °C)	Positive Control 1	5.00	5.50	$R = \log_{10} C - \log_{10} T$ $R = 5.00$	%99.999
	Positive Control 2	6.00			

TEST	Experiments	(Log ₁₀ TCID ₅₀)	Mean	The average reduction in the viral load	The average reduction in the viral load
	Test 1	0.5	-		

In the table, R is the reduction in viral load, C is an average of TCID₅₀ of the positive control groups, and T is the TCID₅₀ values of the test groups. The residence time of the air was 1.44 seconds at the air flow rate of 0.6 m³/h in the heater with a cross-section area of 4x4 cm² and length of 15 cm. During this time, the air temperature increased from 20 °C to 150°C or 220 °C.

An analytical model based on the rate law for a first-order reaction and the Arrhenius equation was used to determine the temperature dependence of the rate constant and estimate the time required to inactivate SARS-CoV-2 (Hessling et al., 2020; Yap et al., 2020). The rate constant $k(T)$ for thermal inactivation of both SARS-CoV-2 and SARS-CoV was obtained (Hessling et al., 2020). Using the same data, we calculated the inactivation time 0.320 s for 3 log₁₀ reductions at 150 °C and 0.007 s for 5 log₁₀ reductions at 220 °C in the viral load. The residence time of the virus in the heater is 4.5 times and 205 times longer than the calculated time needed to inactivate the virus at the temperature of 150 °C and 220°C. The approach of using the heater outlet temperature as a reference is reasonable since the estimated times for inactivation of the viruses are a fraction of a second and much smaller the residence time for the selected temperatures.

4 | CONCLUSION

Our results show that high-temperature is very effective in inactivating aerosolized SARS-CoV-2 within a second. It can be implemented primarily during winter, just by increasing the heater’s temperature to 150°C or above for a fraction of a second to provide 3 log₁₀ reductions in the viral load of SARS-CoV-2 in air. It has the potential to be used in houses, hospitals, shopping centers, HVAC (heating, ventilating, and air conditioning) systems, and public transport vehicles during winter.

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AUTHOR CONTRIBUTIONS

Conceptualization, MC, AYC, and ET; Methodology, MC, AYC, SB and CS; Formal Analysis, MC, CS, DK; Investigation, MC, AYC, SB, DK, CS, and ET; Data Curation, MC and CS; Writing – Original Draft Preparation, MC, CS, SB, DK; Writing – Review and Editing, ACY and ET.

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