RESEARCH, DISCOVERY & INNOVATION

Water & Energy Sustainable Technology Center

NON-GLP STUDY REPORT

STUDY TITLE

Effectiveness of Steam for the Inactivation of Infectious SARS-CoV-2 on Stainless Steel (Non-Porous) and Non-Glazed Ceramic Tile (Porous)

TEST DEVICE

Eagle/US6100 Steam Cleaner

STUDY SCIENTISTS

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STUDY SPONSOR

U.S. Steam

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RESEARCH, DISCOVERY & INNOVATION

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STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Effectiveness of Steam for the Inactivation of Infectious SARS-CoV-2 on Stainless Steel (Nonporous) and Non-Glazed Ceramic Tile (Porous)

TEST DEVICE IDENTITY

Test Device: Eagle/US6100 Steam Cleaner

TEST SURFACES

- Stainless steel (Type 304, unidirectional finish, 2-in^2): Hard, nonporous
- Non-glazed tile (4-in^2): Hard, porous
- Control replicates (no stream treatment) evaluated in replicates of three (3)
- Test replicates (steam-treated) evaluated in replicates of five (5) per contact time, per surface type

REPORT DATE: 13 October 2021

TEST PARAMETERS

Virus: SARS-CoV-2, Isolate USA-WA1/2020 (BEI Resources NR-52281)

Inoculum Volume Tested: 20 uL per carrier

Inoculum Area: 1-in^2

Carrier Drying Conditions: 21 - 22 degrees C, 55 - 65% RH

Exposure Times: 1 second, 3 seconds, 5 seconds

Organic Soil Load: 5% Fetal Bovine Serum (FBS) soil load

Carrier Harvest Medium: 0% FBS Minimal Essential Medium (MEM); 1 mL per carrier

Test Medium: 5% FBS MEM

Indicator Cell Cultures: Vero E6 Cells (ATCC® CRL-1586)

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Experiment Design

Test Device Preparation

On the day of testing, the reservoir of the steam device was filled with 3L of tap water dechlorinated by sequential passage through two granular activated carbon filters. The device was powered on, which facilitated heating of the test water and pressurization of the system. The device was considered as ready-to-use when the pressure gauge displayed a range of 4 to 5 bar. The steam delivery hose was outfitted with the 3" round nylon brush attachment for the test.

Carrier Preparation

On the day prior to testing, stainless steel and non-glazed tile carriers were placed into sterile Petri dishes and sanitized by spraying with 95% ethanol. On the day of testing, 18 carriers per each surface type were inoculated with 20 uL of test virus stock (amended with 5% FBS organic soil), with spreading over an area of 1-in^2 using a sterile, bent pipette tip. The carriers were then dried within a Class II B2 biosafety cabinet with the Petri dish lids slightly ajar (21 - 22 degrees C, 55 - 65% RH).

Test Procedure and Assay

Just prior to each carrier test exposure, the steam delivery nozzle was activated and primed for 5 to 8 seconds to clear the line of excess liquid water. The nozzle was then placed over the center of each test carrier to ensure direct delivery of steam over the 1-in^2 inoculum zone, and held for the specified study contact time (1 second, 3 seconds, or 5 seconds). The carrier distance from the nozzle exit during steam application was approximately one inch. Following the steam treatment, each carrier was washed 4-5 times using 1 mL of 0% FBS MEM and treated using a cell scraper to further facilitate detachment of viruses. Control carriers (performed in triplicate per surface type) were not subjected to steam treatment, and were harvested in the same manner as the test carriers. The virus suspensions from the control and test carriers underwent serial dilutions (1:10 in 0% FBS MEM) that were assayed in replicates of six for infectious viruses using Vero E6 host cells prepared in 96-well trays. Cultures were scored periodically over 10 days for the absence/presence of cytopathic effects (CPE), cytotoxicity and viability. The CPE associated with SARS-CoV-2 was visually evidenced under the microscope by the presence of cell rounding/aggregation, detachment from the monolayer, and eventual regrowth of the monolayer.

RESULTS

Table 1. Inactivation of SARS-CoV-2 using steam on stainless steel coupons[a,b]

Carrier ID	Contact Time	Virus Recovery (Log_{10} per Carrier)	Mean Recovery (Log_{10})	Log_{10} Reduction	Percent Reduction
Control 1	N.A.	3.00	2.94 ± 0.25	N.A.	N.A.
Control 2		2.67			
Control 3		3.17			
Test 1	1 second	≤ 0.50	≤ 1.07 ± 0.36	≥ 1.88	≥ 98.5%
Test 2		1.00			
Test 3		1.17			
Test 4		1.50			
Test 5		1.17			
Test 1	3 seconds	0.67	≤ 0.60 ± 0.09	≥ 2.34	≥ 99.6%
Test 2		0.67			
Test 3		≤ 0.50			
Test 4		≤ 0.50			
Test 5		0.67			
Test 1	5 seconds	≤ 0.50	≤ 0.50 ± 0.00	≥ 2.44	≥ 99.7%
Test 2		≤ 0.50			
Test 3		≤ 0.50			
Test 4		≤ 0.50			
Test 5		≤ 0.50			

[a]N.A.: Not applicable.

[b]"≤": No viral CPE observed; therefore, infectious virus levels at or below the limit of detection (0.50 log_{10}).

RESULTS

Table 2. Inactivation of SARS-CoV-2 using steam on non-glazed ceramic tiles[a]

Carrier ID	Contact Time	Virus Recovery (Log_{10} per Carrier)	Mean Recovery (Log_{10})	Log_{10} Reduction	Percent Reduction
Control 1	N.A.	2.83	2.83 ± 0.17	N.A.	N.A.
Control 2		2.67			
Control 3		3.00			
Test 1	1 second	2.17	1.93 ± 0.25	0.90	86.6%
Test 2		2.00			
Test 3		2.00			
Test 4		2.00			
Test 5		1.50			
Test 1	3 seconds	1.33	1.70 ± 0.52	1.13	88.0%
Test 2		1.50			
Test 3		2.33			
Test 4		2.17			
Test 5		1.17			
Test 1	5 seconds	0.83	1.47 ± 0.43	1.37	94.2%
Test 2		1.50			
Test 3		1.33			
Test 4		1.67			
Test 5		2.00			

[a]N.A.: Not applicable.

CONCLUSIONS

This study was conducted to assess the antiviral effectiveness of steam applied at three contact times (1 second, 3 seconds, and 5 seconds) against SARS-CoV-2 dried onto stainless steel (hard, non-porous) and non-glazed ceramic tiles (hard, porous). SARS-CoV-2 was more efficiently inactivated on stainless steel, with infectious virus levels reduced to levels below detection on all five test replicates following 5 seconds of exposure to steam (> 99% reduction). In contrast, infectious SARS-CoV-2 was detected on all test replicates for each of the three contact times following steam treatment of porous, non-glazed ceramic tile. However, a reduction of > 90% reduction was still achieved on the tiles during the five second contact time. The difference in effectiveness may be attributed to the porosity of the ceramic tile, which acted in part to shield viruses which had penetrated the pores from direct contact with the steam.