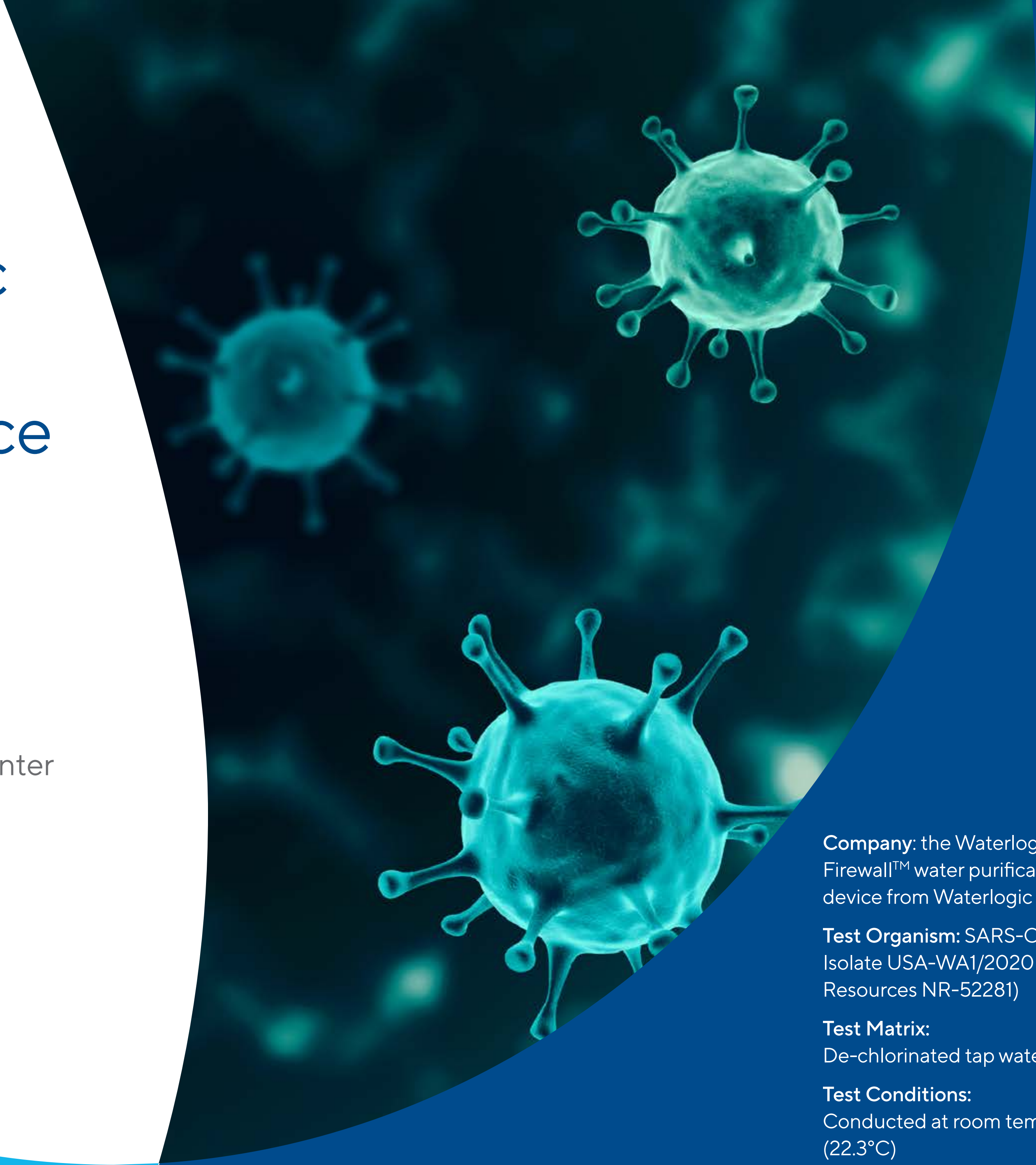
Firewall® SARS-CoV-2 Test Study Report

Reduction of SARS-CoV-2 by the Waterlogic Firewall® water purification device



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Company: the Waterlogic Firewall™ water purification device from Waterlogic

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

Test Matrix:
De-chlorinated tap water

Test Conditions:
Conducted at room temperature (22.3°C)

Experimental Design

Two separate Waterlogic Firewall® water purification devices tested their ability to inactivate SARS-CoV-2 in tap water in two separate tests.

1. Placed 1 liter of sterile deionized water in the device reservoir. Plugged in and turned on the device and dispensed 0.75 liters through the device. The collected water was discarded, and the reservoir was emptied. This step primed the device, removing any air in the system, and filled the internal reservoir which holds 0.39 liters.
2. Added 1 ml of virus stock, containing approximately 1.0×10^8 TCID50 SARS-CoV-2, Isolate USA-WA1/2020 (BEI Resources NR-52281), to a 1-liter bottle of sterile de-chlorinated tap water and mixed thoroughly.
3. Placed the entire liter bottle in the device reservoir.
4. Collected three 30-ml samples from the reservoir in sterile conical tubes (Influent A, B, and C samples).
5. Dispensed 0.5 liters into discard bucket to flush out the internal 0.39-liter reservoir to ensure that water with virus was being dispensed for the test.
6. Dispensed three effluent samples of 30-ml each in sterile 50-ml conical tubes (Effluent A, B, and C samples).
7. Inoculated interior of dispenser faucet nozzle with a sterile polyester swab dipped into virus stock, containing 6.3×10^6 TCID50/ml SARS-CoV-2. Swabbed inside of the faucet tip. This included inserting the swab up inside as far as it would go and twisting it back and forth. (The head of the Firewall housing is designed and contoured so that some UV chases the water out of the dispenser faucet. At a ½-inch distance outside of the faucet nozzle, UV dose is measured at 12 uW-Sec/cm2).
8. Dispensed 10-ml volume of water from the unit to allow for exposure of the nozzle to the UV light.
9. Dipped a sterile polyester swab into a 5-ml snap cap tube, containing 1 ml of sterile PBS, and used this to swab the interior of the faucet nozzle. Replaced the swab back into the tube and broke off the excess wooden handle so that the swab would fit inside the tube.
10. Vortexed this tube for 30 seconds to elute the virus from the swab and then aseptically removed and discarded the swab. This solution was the "Nozzle" sample.

Samples collected and assayed:

Device #1 – Influent A	Device #2 - Influent A
Influent B	Influent B
Influent C	Influent C
Effluent A	Effluent A
Effluent B	Effluent B
Effluent C	Effluent C
Nozzle	Nozzle

11. Virus concentrations for each neutralized sample were quantified, using the Reed-Muench method (Payment and Trudel 1993), to determine the tissue culture infectious dose that affected 50% of the wells (TCID50). The assay was performed in 96-well cell culture plates, containing monolayers of Vero E-6 cell monolayers (ATCC# CRL-1586). Prior to the assay, the Vero E-6 cells were gently rinsed twice with minimal essential media (MEM). The 96-well plates were then inoculated with the diluted samples (6 wells inoculated with 100 microliters each per dilution). Flasks, containing 1-ml (in 25 cm2 flasks) and 10-ml samples (in 75 cm2 flasks), were also included to lower the limit of detection of the assay. The plates/flasks were incubated in an atmosphere of 5% CO_2 for 1 hour at 37 °C to allow the virus particles to adsorb to the cells.
Note: Each 96-well plate also included at least 6 negative control wells, containing cells only (no virus) with 100 microliters of MEM added.

12. Following this incubation period, 85 microliters of MEM, containing 2% fetal bovine serum (FBS), were added to each of the 96 wells, 7 ml were added to the 25 cm2 flasks, and 20 ml were added to the 75 cm2 flasks. The plates/flasks were then incubated in an atmosphere of 5% CO_2 for 7 days at 37 °C.

13. The cells were observed daily for viral cytopathic effects (CPE), using an inverted microscope. The inoculated cells were compared to the negative control cells in the same 96-well plate to differentiate CPE from un-inoculated cells. Negative control flasks were also included in the assay. Any CPE that was observed within 24 hours of incubation was considered to be caused by cytotoxicity (caused by sensitivity of the cells to the tap water), since CPE caused by SARS-CoV-2 typically requires ≥ 2 days. Wells positive for CPE, following 2 days, were considered positive for viral growth.
Note: No CPE was observed in any of the negative control wells.

14. After the incubation period, the TCID50/sample was determined. Six wells per dilution were used to ensure adequate precision of the assay. The greatest dilution in which 50% or higher of the wells were positive was used to determine the virus TCID50/coupon, following the method described by Payment and Trudel (1993).

15. The data were reported as the logarithmic reduction, using the formula $-\log_{10}$ (Neff/Ninf) where Ninf was the average concentration of the recovered SARS-CoV-2 from the influent samples, and Neff was the concentration of the recovered SARS-CoV-2 in the effluent samples.

16. A Student's t-test was used to statistically compare the virus recovered from the influent (no UV) and the effluent (treated with UV) samples. The reductions were considered to be statistically significant if the resultant P value was ≤ 0.05.

17. The average percent reduction was also calculated. The relationship between $\log_{10}$ reduction and percent reduction is illustrated in Table 1 below.

Table 1. Log_{10} removal versus percent reduction.

Log_{10} Removal	Percent Reduction (%)
1	90
2	99
3	99.9
4	99.99
5	99.999
6	99.9999

References

Payment P, Trudel M. (1993) Isolation and identification of viruses. In Methods and Techniques in Virology. Payment P, Trudel M (eds.), pp. 32–33. New York: Marcel Dekker Inc.

Results

The results of the tests are shown below in Tables 2 and 3.

Table 2. Reduction of SARS-CoV-2 by the Waterlogic Firewall® water purification device.

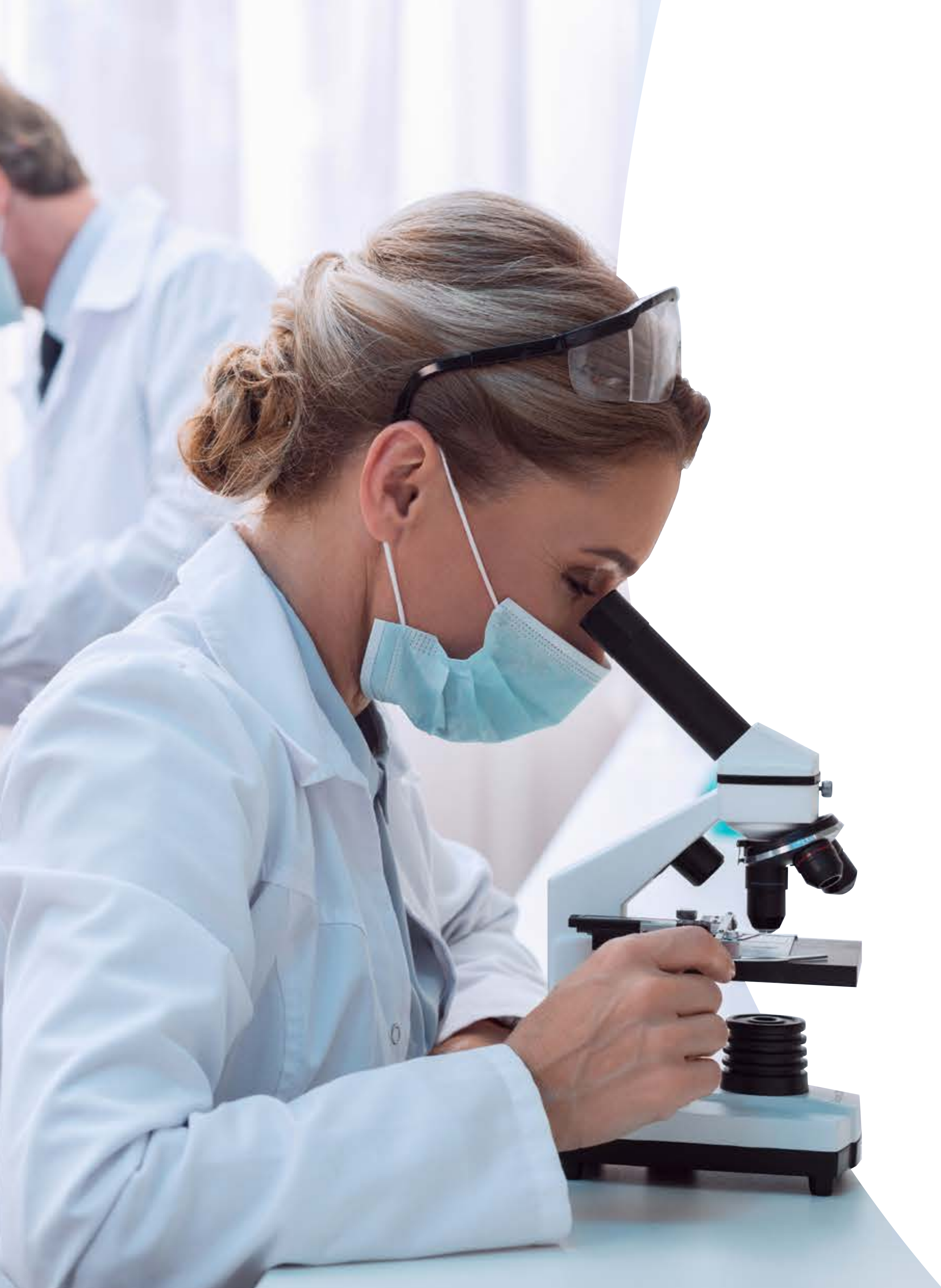
Device	Log_{10} Reduction* Per Effluent Sample	Mean Log_{10} Reduction ± SD	Mean Percent Reduction
	> 5.67		
Unit 1	> 5.67	>5.67† ± 0.00	>99.99979
	> 5.67		
	> 5.89		
Unit 2	> 5.89	>5.89† ± 0.00	>99.99987
	> 5.89		

* The average of the three influent samples was 1.86×105 TCID50/ml and 3.10×105 TCID50/ml for unit #1 and unit #2, respectively. The log_{10} reductions in effluent samples were calculated using these values.
SD Standard deviation
† Reductions in the treated samples were statistically significant (P ≤ 0.05) in comparison to the influent samples (no UV treatment).

Table 3. Reduction of SARS-CoV-2 on the dispenser faucet nozzle of the Waterlogic Firewall® water purification device.

Device	Estimated Log_{10} Reduction*	Percent Reduction
Unit 1	3.20 to 3.70	99.94 to 99.98
Unit 2	> 3.20 to > 3.70	> 99.94 to > 99.98

* An estimated 100 microliters of the inoculum virus stock containing 6.3×105 TCID50 was transferred from the swab to the dispenser nozzle. Based on an estimated recovery efficiency of 10% (1.0 log_{10} loss) to 31.6% (0.5 log_{10} loss) of SARS-CoV-2 from the nozzle using a swab dipped in PBS, an estimated 6.3×104 to 2.0×105 virus would be recovered without any UV exposure. The log_{10} and percent reductions in the nozzle samples were calculated using these estimated value ranges.



Discussion

No infectious SARS-CoV-2 particles were recovered from any of the effluent water samples, following treatment by either of the two Waterlogic Firewall® water purification devices tested.

Therefore, the virus concentration was below the limit of detection of the assay (3.98×10^{-1} $TCID_{50}$/ml) in all effluent samples. This was equivalent to a > 5.67 log10 reduction for the test with unit #1 and a > 5.89 log10 reduction with unit #2. These reductions were statistically significant in comparison to the influent samples ($P = 1.4 \times 10^{-5}$ and 1.9×10^{-7}, respectively).

In addition, the approximate 12 uW-Sec/cm2 UV dose at the Waterlogic Firewall® faucet nozzle resulted in a reduction of infectious SARS-CoV-2 inoculated onto the nozzle itself. This test was performed to simulate an ill individual, coughing or sneezing in close proximity to the device. An estimated 3.20 to >3.70 log_{10} reductions were observed on the nozzle. However, a portion of the virus on the nozzles could have been washed away in the 10-ml samples that were discarded as part of the sample collection process.

Better thinking, better water, better for you, better for the planet™

At Waterlogic, everything starts with the way we think about water. Behind every drop of Waterlogic water are years of knowledge, innovation, and experience to deliver purified, great-tasting water in the safest and most sustainable way.

And because we design, manufacture, distribute, install, and service our own water dispensers, you can enjoy unparalleled product quality, including a variety of consumables and accessories and highly responsive Total Care service that is second to none.

Contact us today to learn more about Waterlogic and find out which solution is right for you.

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