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Environmental Persistence and Disinfection of Lassa Virus

Appendix

Wastewater Characterization

Approximately 1 L of primary influent wastewater was aseptically collected from a wastewater treatment plant in northern Indiana, United States, and stored at -80°C before being shipped overnight on ice to Rocky Mountain Laboratories (RML). All physiochemical characterization and chlorine demand testing were conducted at the University of Notre Dame. Appendix Table 1 describes the physiochemical characteristics of the influent wastewater used in the following experiments.

Appendix Table 1. Composition of tested wastewater

Measurements	Test results
pH	6.16
Chemical oxygen demand, mg/L	279
Ammonia, mg/L	29
Nitrate, mg/L	5.0
Phosphorus, mg/L	9.2
Total suspended solids, mg/L	134

Appendix Table 2. Recorded TCID₅₀ values for 2 Lassa virus strains on high-density polyethylene over time*

Time, d	Josiah strain	Sauerwald strain
0	868,340.4 (443,997.8)	652,379.4 (462,663.2)
1	22,270.8 (12,317.4)	4,479.2 (1,598.1)
2	694.4 (1,130.9)	592.4 (553.4)
3	6.3 (0)†	632.5 (0)
4	6.3 (0)†	6.3 (0)†
5	6.3 (0)†	6.3 (0)†

*Values are no. (SD). TCID₅₀, 50% tissue culture infectious dose.

†Values are at the limit of detection for the TCID₅₀ assay.

Appendix Table 3. Recorded TCID₅₀ values for 2 Lassa virus strains on stainless steel over time*

Time, d	Josiah strain	Sauerwald strain
0	868,340.4 (443,997.8)	355,655.9 (0)
1	22,270.8 (12,317.4)	13,188.5 (19,379.1)
2	694.4 (1,130.9)	1,124.7 (0)
3	6.3 (0)†	229.3 (349.2)
4	6.3 (0)†	6.3 (0)†
5	6.3 (0)†	6.3 (0)†

*Values are no. (SD). TCID₅₀, 50% tissue culture infectious dose.

†Values are at the limit of detection for the TCID₅₀ assay.

Appendix Table 4. Recorded TCID₅₀ values for 2 Lassa virus strains in deionized water over time*

Time, d	Josiah strain	Sauerwald strain
0	1,834,299.5 (1,300,873.0)	457,487.3 (487,482.5)
1	708,227.6 (252,682.4)	223,961.2 (79,905.2)
2	1,492,835.1 (1,485,653)	316,227.8 (0)
3	398,265.6 (142,093.7)	111,354.0 (61,586.9)
4	316,227.8 (0)	62,619.0 (34,632.9)
5	626,189.7 (346,328.7)	70,822.8 (25,268.2)

*Values are no. (SD). TCID₅₀, 50% tissue culture infectious dose/mL.

Appendix Table 5. Recorded TCID₅₀ values for 2 Lassa virus strains in wastewater over time*

Time, d	Josiah strain	Sauerwald strain
0	1,518,852.9 (449,339.8)	244,151.8 (124,839.2)
1	239,525.2 (280,422.1)	49,801.9 (440,420.2)
2	39,826.6 (14,209.4)	12,483.9 (12,483.9)
3	95,942.7 (83,987.5)	1,980.2 (1,095.2)
4	8,856.2 (7,828.0)	544.2 (394.8)
5	1,706.1 (1,493.5)	11.14 (6.2)

*Values are no. (SD). TCID₅₀, 50% tissue culture infectious dose/mL.

Appendix Table 6. Recorded TCID₅₀ values for 2 Lassa virus strains in 0 mg/L sodium hypochlorite over time*

Time, min	Josiah strain	Sauerwald strain
0	1,252,379.4 (692,657.4)	125,237.9 (69,265.7)
0.3	1,678,965.8 (1,670,888.2)	125,237.9 (69,265.7)
1	77,087.0 (61,282.1)	141,645.5 (50,536.5)
5	704,264.7 (389,509.9)	116,011.3 (82,274.4)
15	796,531.2 (284,187.5)	79,653.1 (28,418.7)
30	447,922.4 (159,810.4)	20,000.0 (0)

*Values are no. (SD). TCID₅₀, 50% tissue culture infectious dose/mL.

Appendix Table 7. Recorded TCID₅₀ values for 2 Lassa virus strains in 1 mg/L sodium hypochlorite over time*

Time, min	Josiah strain	Sauerwald strain
0	1,252,379.4 (692,657.4)	96,060.7 (28,418.7)
0.3	116,011.3 (82,274.4)	3,504.5 (2,846.4)
1	48,830.4 (24,967.8)	2,063.0 (1,463.1)
5	3,037.7 (898.7)	960.6 (284.2)
15	560.1 (495.1)	366.9 (260.2)
30	6.3 (0)†	6.3 (0)†

*Values are no. (SD). TCID₅₀, 50% tissue culture infectious dose/mL.

†Values are at the limit of detection for the TCID₅₀ assay.

Appendix Table 8. Recorded TCID₅₀ values for 2 Lassa virus strains in 5 mg/L sodium hypochlorite over time*

Time, min	Josiah strain	Sauerwald strain
0	1,252,379.4 (692,657.4)	540,188.9 (159,810.4)
0.3	1,124.7 (0)	632.5 (0)
1	632.5 (0)	170.8 (50.5)
5	6.3† (0)	41.7 (61.3), 112.5 (0)‡
15	41.7 (63.1), 112.5 (0)‡	6.3 (0)†
30	6.3 (0)†	6.3 (0)†

*Values are no. (SD). TCID₅₀, 50% tissue culture infectious dose/mL.

†Values are at the limit of detection for the TCID₅₀ assay.

‡Indicates ≥1 values were at the limit of detection; values in parentheses show SD when those values were excluded.

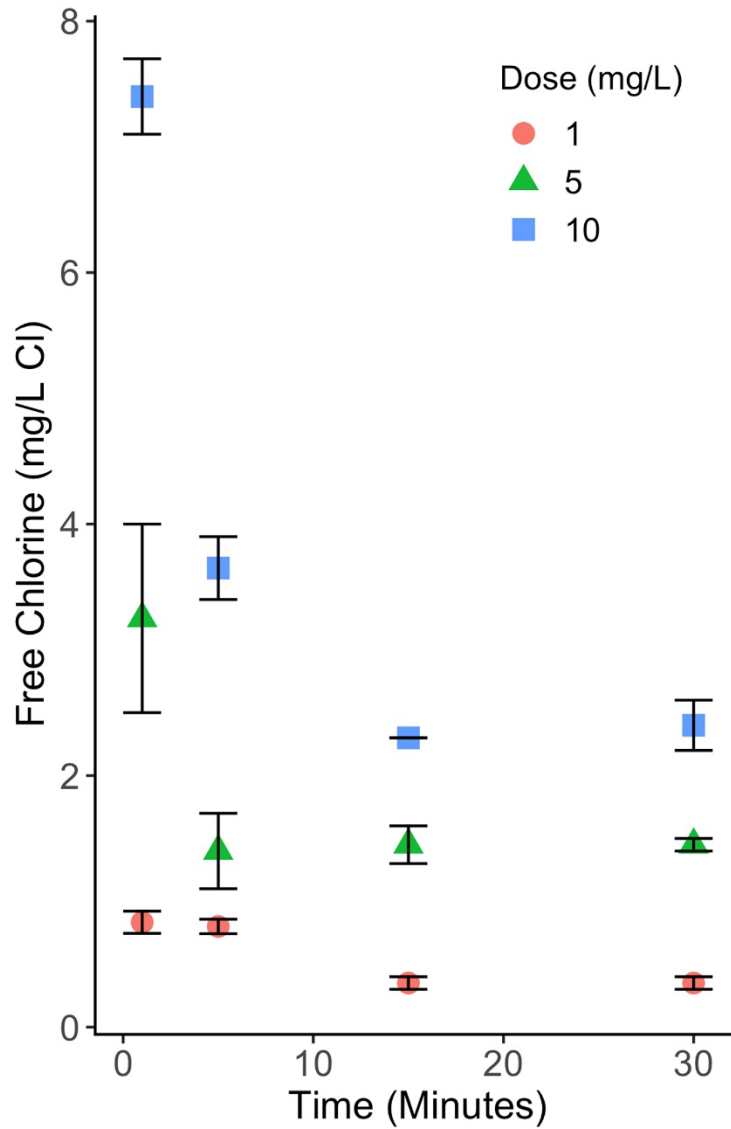
Appendix Table 9. Recorded TCID₅₀ values for 2 Lassa virus strains in 10 mg/L sodium hypochlorite over time*

Time, min	Josiah strain	Sauerwald strain
0	2,063,004.8 (1,463,069.4)	222,708.0 (123,173.8)
0.3	632.5 (0)	632.5 (0)
1	540.2 (159.8)	6.3 (0)†
5	6.3 (0)†	6.3 (0)†
15	77.1 (61.3), 112.5 (0)‡	6.3 (0)†
30	6.3 (0)†	6.3 (0)†

*Values are no. (SD). TCID₅₀, 50% tissue culture infectious dose/mL.

†Values are at the limit of detection for the TCID₅₀ assay.

‡Indicates ≥1 values were at the limit of detection; values in parentheses show SD when those values were excluded.



Appendix Figure. Free Chlorine Concentrations for 1, 5, and 10 mg/L of free chlorine as sodium hypochlorite. The y-axis shows the free chlorine concentration as mg/L of Cl determined by the HACH D900 Colorimeter. The x-axis shows the time in minutes. Each data point represents the mean of 3 replicates, and the error bars show the standard deviation.