## **Supplementary Material**

## Quantifying the viral reduction achieved using ash and sand as handwashing agents

Winnie Zambrana<sup>1</sup>, Jingyan Tong<sup>1</sup>, Claire E. Anderson<sup>1</sup>, Alexandria B. Boehm<sup>1\*</sup>, Marlene K. Wolfe<sup>1,2\*</sup>

 Department of Civil & Environmental Engineering, Stanford University, 473 Via Ortega, Stanford, CA, USA, 94305
 Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA, 30322

\*Corresponding authors Alexandria B. Boehm, aboehm@stanford.edu, 650-724-9128 Marlene K. Wolfe, marlene.wolfe@emory.edu, 617-583-2401

**Tables S1-S5** show the results for the statistical analysis tests. For the Kruskal-Wallis test, the P-value is reported to demonstrate that a significant difference was found among the LRVs resulting from the handwashing methods by testing the null hypothesis that the data sets come from the same distribution using statistical significance of p=0.05. For the Conover-Iman test, the test statistic and P-value adjusted are both reported. The P-value adjusted indicated whether a statistically significant difference was found between each pairwise comparison using statistical significance of p=0.025. The sign of the test statistic is used to indicate the directionality of the differences (i.e., which of the two conditions in a pairwise comparison has a greater LRV).

**Table S1.** Phi6 Results Summary - Experimental results and Shapiro-Wilk and Kruskal-Wallis tests results

Handwashing Condition	Number of Samples/ Condition	Median Log Reduction	Min Log Reduction	Max Log Reduction	Shapiro- Wilk P-value	Kruskal- Wallis P- value
Water Only (20 Sec)		3.5	1.7	5.6	7.14 x 10 <sup>-1</sup>	
Soap and Water (20 Sec)		4.8	1.5	6.6	4.72 x 10 <sup>-1</sup>	
Ash and Water (5 Sec)		2.8	0.4	5.2	2.81 x 10 <sup>-1</sup>	

Handwashing Condition	Number of Samples/ Condition	Median Log Reduction	Min Log Reduction	Max Log Reduction	Shapiro- Wilk P-value	Kruskal- Wallis P- value
Ash and Water (20 Sec)	15	3.6	2.0	5.9	2.15 x 10 <sup>-1</sup>	1.66 x 10 <sup>-2</sup> *
Sand and Water (5 Sec)		2.7	0.5	6.2	6.69 x 10 <sup>-1</sup>	
Sand and Water (20 Sec)		3.7	2.0	5.9	8.45 x 10 <sup>-1</sup>	

**Note:** \*Statistically significant (p <5.00 x 10<sup>-2</sup>).

## Table S2. Phi6 Conover-Iman Test Results - Comparison of LRVs between conditions

Handwashing Condition	Water Only (20 Sec)	Soap and Water (20 Sec)	Ash and Water (5 Sec)	Ash and Water (20 Sec)	Sand and Water (5 Sec)
Water Only (20 Sec)	-				
Soap and Water	-2.20		]		
(20 Sec)	(2.30 x 10 <sup>-1</sup> )	-			
Ash and Water (5 Sec)	0.99	3.18			
	$(1.00 \times 10^{\circ})$	(1.52 x 10 <sup>-2</sup> )*	-		
Ash and Water (20 Sec)	-1.01	1.19	-2.00		
	$(1.00 \times 10^{0})$	$(1.00 \times 10^{0})$	(3.66 x 10 <sup>-1</sup> )	-	
Sand and Water	1.07	3.27	0.08	2.08	
(5 Sec)	$(1.00 \times 10^{0})$	(1.17 x 10 <sup>-2</sup> )*	$(1.00 \text{ x } 10^{\circ})$	(3.02 x 10 <sup>-1</sup> )	-
Sand and Water	-0.42	1.78	-1.41	0.59	-1.49
(20 Sec)	$(1.00 \times 10^{\circ})$	$(5.94 \times 10^{-1})$	$(1.00 \times 10^{\circ})$	$(1.00 \times 10^{0})$	$(1.00 \times 10^{\circ})$

Notes: Values indicate Connover-Iman test statistic and in parenthesis the P-value adjusted for Bonferroni correction; Positive test statistic value indicates Column Condition LRV > Row Condition LRV, negative test statistic value indicates Column Condition LRV < Row Condition LRV; \*statistically significant ( $p < 2.50 \times 10^{-2}$ ).

**Table S3.** MS2 experimental results summary and results of Shapiro-Wilk and Kruskal-Wallis tests

Handwashing Condition	Number of Samples/ Condition	Median Log Reduction	Min Log Reduction	Max Log Reduction	Shapiro-Wilk P-value	Kruskal-Wallis P-value
Water Only (20 Sec)		2.8	2.4	4.2	7.64 x 10 <sup>-3</sup> *	

Handwashing Condition	Number of Samples/ Condition	Median Log Reduction	Min Log Reduction	Max Log Reduction	Shapiro-Wilk P-value	Kruskal-Wallis P-value
Soap and Water (20 Sec)		2.7	2.0	4.0	2.94 x 10 <sup>-1</sup>	
Ash and Water (5 Sec)	19	2.0	1.0	3.0	9.61 x 10 <sup>-1</sup>	3.61 x 10 <sup>-10</sup> *
Ash and Water (20 Sec)		2.6	1.6	3.5	5.34 x 10 <sup>-1</sup>	5.01 x 10
Sand and Water (5 Sec)		1.8	1.1	2.6	9.75 x 10 <sup>-1</sup>	
Sand and Water (20 Sec)		2.4	1.3	3.5	1.70 x 10 <sup>-1</sup>	

**Note:** \*Statistically significant ( $p < 5.00 \times 10^{-2}$ )

Table S4. MS2	Conover-Iman	Test Results -	Comparison	between conditions
---------------	--------------	----------------	------------	--------------------

Handwashing Condition	Water Only (20 Sec)	Water and Soap (20 Sec)	Ash and Water (5 Sec)	Ash and Water (20 Sec)	Sand and Water (5 Sec)
Water Only (20 Sec)	-		_		
Soap and Water (20 Sec)	0.80 (1.00 x 10 <sup>0</sup> )	-			
Ash and Water (5 Sec)	6.50 (1.88 x 10 <sup>-8</sup> )*	5.71 (7.71 x 10 <sup>-7</sup> )*	-		_
Ash and Water (20 Sec)	3.05 (2.15 x 10 <sup>-2</sup> )*	2.25 (1.97 x 10 <sup>-1</sup> )	-3.45 (5.95 x 10 <sup>-3</sup> )*	-	
Sand and Water (5 Sec)	7.83 (2.65 x 10 <sup>-11</sup> )*	7.03 (1.44 x 10 <sup>-9</sup> )*	1.33 (1.00 x 10 <sup>0</sup> )	4.78 (4.15 x 10 <sup>-5</sup> )*	-
Sand and Water (20 Sec)	3.61 (3.45 x 10 <sup>-3</sup> )*	2.81 (4.35 x 10 <sup>-2</sup> )	-2.89 (3.49 x 10 <sup>-2</sup> )	0.56 (1.00 x 10 <sup>0</sup> )	-4.22 (3.84 x 10 <sup>-4</sup> )*

Notes: Values indicate Connover-Iman test statistic and in parenthesis the P-value adjusted for Bonferroni correction; Positive test statistic value indicates Column Condition LRV > Row Condition LRV, negative test statistic value indicates Column Condition LRV < Row Condition LRV; \*statistically significant ( $p < 2.50 \times 10^{-2}$ ).

**Table S5.** Results of comparison of LRVs between bacteriophages (MS2 LRVs against Phi6LRVs) per condition using Kruskal-Wallis and Conover-Iman tests

Handwashing Condition	Kruskal- Wallis P-value	Conover- Iman Test Statistic	Conover- Iman P- value Adjusted
Water Only (20 Sec)	3.29 x 10 <sup>-2</sup> *	-2.26 (MS2 < Phi6)	1.53x 10 <sup>-2</sup> **
Soap and Water (20 Sec)	1.68 x 10 <sup>-4</sup> *	-4.90 (MS2 < Phi6)	1.31x 10 <sup>-5</sup> **
Ash and Water (5 Sec)	1.23 x 10 <sup>-1</sup>	-	-
Ash and Water (20 Sec)	1.05 x 10 <sup>-3</sup> *	-3.93 (MS2 < Phi6)	2.13 x 10 <sup>-4</sup> **
Sand and Water (5 Sec)	7.41 x 10 <sup>-2</sup> *	-	-
Sand and Water (20 Sec)	2.53 x 10 <sup>-4</sup> *	-4.67 (MS2 < Phi6)	2.55 x 10 <sup>-5</sup> **

**Notes:** \*Statistically significant ( $p < 5.00 \times 10^{-2}$ ), \*\*P-value adjusted for Bonferroni correction, statistically significant ( $p < 2.50 \times 10^{-2}$ ).