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Evaluation of MS-2 Reduction by UV Water Box Water Disinfection

Report

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Project Summary

The Water Box device for disinfection of potable water by UV light was tested for anti-viral efficacy using MS-2 *Escherichia coli* (*E. coli*) bacteriophage. Performance was tested with a reservoir filled with 4.1 L of distilled water that was inoculated with approximately $1x10^7$ MS-2 viral particles and contaminated with 1.7 ppm 4-hydroxybenzoic acid (PHBA) to reduce UV254 transmission to 80 %. A 100 mL sample of contaminated water was collected prior to UV processing, and a second sample was collected after irradiation. A third sample was collected to measure the UV254 transmission of the challenge water for each test. The Water Box UV lamp was powered by the provided power supply for all irradiated tests. Samples were diluted and plated on Tryptic Soy Agar (TSA) plates with *E. coli* inoculum for MS-2 enumeration.

Procedures and Data

UV Transmission Adjustment

The test protocol specified that the challenge water be modified with the addition of PHBA to reduce UV254 transmission to 80%. An Ultrospec 1000 spectrophotometer was used to measure the transmission of 254 nm light over a 1 cm path length. Dilutions of a 500 ppm solution established that 1.7 ppm PHBA would reduce transmission to approximately 80 %.

Challenge Water Preparation:

The MS-2 inoculum was prepared as in the previous evaluation. Plaque counts of the filtrate determined that the viral density was $4.6x10^{10}$ PFU/mL. This culture was used to inoculate separate volumes of challenge water for each of the prescribed tests. Sufficient PHBA concentrate was added to bring the concentration to 1.7 ppm. The challenge water used for each test was prepared immediately prior to use.

Anti-Viral Challenges:

Test 1:

To begin, The Water Box was filled with 4.1 L of challenge water. Next, a 100 mL "Influent" sample was collected in a sterile bottle via the Water Box outlet. The water was then left untreated for 10 minutes. Finally, a 100 mL "Effluent" sample for plating and a 20 mL sample for UV254 transmission measurement were collected at the outlet. The analyst then began preparation of the second test while a second analyst performed the UV254 measurement, and then diluted and plated the two 100 mL samples on TSA plates with soft agar and an *E. coli* inoculum. Results are presented in Table 1.

Table 1: No Treatment Control Test Results

Test #	Duration (s)	UV254 Transmission (%)	Test Volume (L)	Initial Viral Load	Post Treatment Viral Load	Log Reduction
1	600	76	4.1	$1.8x10^7$	$3.3x10^7$	-0.3

Test 2:

To begin, The Water Box was filled to with 4.1 L of challenge water. Next, a 100 mL "Influent" sample was collected in a sterile bottle via the Water Box outlet. The water was then treated by UV light for 120 seconds. Following the treatment phase, a 100 mL "Effluent" sample and a 20 mL UV254 sample were collected at the outlet. The samples were analyzed as in Test 1. Results are presented in Table 2.

Table 2: Results for 120 Second Water Box Test

Test #	Duration (s)	UV254 Transmission (%)	Test Volume (L)	Initial Viral Load	Post Treatment Viral Load	Log Reduction
2	120	79	4.1	$3.1x10^7$	$3.7x10^6$	0.9

Test 3:

To begin, The Water Box was filled to with 4.1 L of challenge water. Next, a 100 mL "Influent" sample was collected in a sterile bottle via the Water Box outlet. The water was then treated by UV light for 180 seconds. Following the treatment phase, a 100 mL "Effluent" sample and a 20 mL UV254 sample were collected at the outlet. The samples were analyzed as in Test 1. Results are presented in Table 3.

Table 3: Results for 180 Second Water Box Test

Test #	Duration (s)	UV254 Transmission (%)	Test Volume (L)	Initial Viral Load	Post Treatment Viral Load	Log Reduction
3	180	79	4.1	$2.2x10^7$	$4.1x10^6$	0.7

Test 4:

To begin, The Water Box was filled to with 4.1 L of challenge water. Next, a 100 mL "Influent" sample was collected in a sterile bottle via the Water Box outlet. The water was then treated by UV light for 120 seconds. Following the treatment phase, a 100 mL "Effluent" sample and a 20 mL UV254 sample were collected at the outlet. The samples were analyzed as in Test 1. Results are presented in Table 4.

Table 4: Results for Repeat of 120 Second Water Box Test

Test #	Duration (s)	UV254 Transmission (%)	Test Volume (L)	Initial Viral Load	Post Treatment Viral Load	Log Reduction
4	120	79	4.1	$3.4x10^7$	$5.7x10^6$	0.8

Test 5:

To begin, the Water Box was filled to with 4.1 L of challenge water. Next, a 100 mL "Influent" sample was collected in a sterile bottle via the Water Box outlet. The water was then treated by UV light for 180 seconds. Following the treatment phase, a 100 mL "Effluent" sample and a 20 mL UV254 sample were collected at the outlet. The samples were analyzed as in Test 1. Results are presented in Table 5.

Table 5: Results for Repeat of 180 Second Water Box Test

Test #	Duration (s)	UV254 Transmission (%)	Test Volume (L)	Initial Viral Load	Post Treatment Viral Load	Log Reduction
5	180	80	4.1	$2.4x10^7$	$2.5x10^7$	0.0