Virus removal test of the Sawyer 7/6BV Filter

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Summary

The ability of the Sawyer 7/6BV filter to remove viruses from surface water was tested according to the USEPA Guide Standard (1986) and Protocol P231 for Testing Microbiological Water Purifiers (the Standard). In accordance with the Standard, the MS2 coliphage (a virus that infects bacteria) was used as a surrogate for human pathogenic viruses; polio virus and rotavirus (a causative agent of waterborne diarrheas). USEPA method 1602 (2001) describes the test protocol.

The Standard requires a minimum reduction of 4 log units, or 99.99% removal of viruses from a test water containing on the order of 1×10^6 viruses per 100 ml. The Sawyer 7/6BV filter reduced the virus concentration by greater than 5.5 log units, or greater than 99.999% (Table 1). As such, the filter exceeds the requirements of the Standard for removal of viruses by more than an order of magnitude.

Table 1. Efficacy of virus removal for the Sawyer 7/6BV filter.

	# detected Initial	**# detected** After filtration	**Units**	**Reduction (%)**	**Reduction (log units)**
Sawyer 7/6BV unit 1	3.9×10^6	12	(plaques/100mL)	**99.9997**	5.5
Sawyer 7/6BV unit 2	3.9×10^6	11	(plaques/100mL)	**99.9997**	5.5

Introduction

This introduction explains in lay terms the test that was conducted. The sections following the introduction are intended to explain the methods, findings, and quality control procedures to a proffesional audience.

Background on bacterial viruses (coliphage):
A virus (MS2 coliphage) that infects bacteria was used in this test. The virus is similar, chemically and physically, to human pathogenic viruses such as the polio virus and the rotaviruses, but it is easier and safer to work with. Thus, it has been accepted as a surrogate for tests using human pathogens. The virus is detected by adding a host bacteria (E. coli) and a solidifying agent to a sample and then pouring the sample into petri dishes. Viral killing of the host bacteria causes the appearance of circular zones of clearing on the plate (Fig. 1) called plaques.

Test procedure:
A suspension of a virus was prepared in sterilized stream water which is referred to as the test water. The test water was passed through the filter using pressurized air (40 psi) to simulate the pressure that would be encountered in a municipal water system. One-hundred ml of test water that passed through the filter was used for enumeration of viruses. Two identical Sawyer 7/6BV filter units were tested. Positive and negative controls were used to evaluate any possible laboratory error. For additional control and for purposes of comparison, a commercially available carbon based water filter that does not make a claim of virus removal was also tested.

Results and conclusion:
The Sawyer 7/6BV filter reduced the virus concentration by 99.9997% (5.53 log units). As such, the two units that were tested exceed the minimum requirements set forth in the Standard for removal of viruses by 1.53 orders of magnitude.

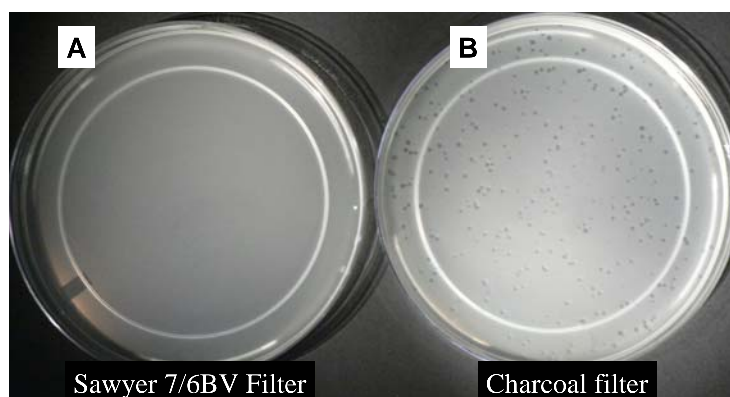

Figure 1. Representative plates for virus enumeration of the Sawyer 7/6BV filter (A) and a commercially available charcoal filter (B). A semi-transparent lawn of bacterial cells covers both plates (150 mm diameter). Circular zones of clearing (plaques) within the lawn on plate B occur where viruses that have passed through the charcoal filter are killing the host bacteria.

Methods

Test organisms: The test organisms and their sources are shown below.

Escherichia coli Famp (ATCC 700891)
Coliphage MS2 (ATCC 15597-B1)

Coliphage and Host Preparation:

Coliphage MS2 host preparation: *E. coli* Famp from a stock culture was inoculated into a 9 ml tryptone dilution tube and allowed to incubate at 36.5 °C for 16 h. 1 ml of the host suspension was inoculated into 50 ml of tryptone broth 4 h before the test to acquire host cells in mid-log phase growth.

Coliphage MS2 preparation: *Coliphage* MS2 (hereafter referred to as phage) from freeze dried ATCC stock was rehydrated and inoculated into a 30 ml culture of host bacteria that had been incubated for 2 h, and then allowed to incubate for an additional 4 h. The cell lysate was removed from the culture by centrifuging at 3600 rpm for 15 min. Phage concentration in the preparation was quantified by adding 1 ml of 10^{-8}, 10^{-9}, and 10^{-10} dilutions of the suspension to each of 5 test tubes (for a total of 15 tubes) containing 3 ml tryptone top agar and 0.1 ml of host cell suspension. Tubes were poured into petri plates and allowed to incubate overnight before plaques were counted. The phage suspension was calculated to have a concentration of 8×10^{10} plaque forming units per ml (pfu/ml).

Test Water: As required by the Standard, the test water was inoculated to a density of approximately 1×10^{7} by adding 0.5 ml of a 10^{-3} dilution of the Coliphage MS2 preparation to one liter of autoclaved, room temperature, water from Rattlesnake Creek (Missoula, MT). Rattlesnake Creek flows out of the Rattlesnake Wilderness Area north of Missoula to its confluence with the Clark Fork River in the city of Missoula. Water was collected near the confluence. Samples of this water were archived and are available upon request. The water is considered representative of normal water used by recreationalists.

Other solutions: Calcium chloride solution, tryptone broth, tryptone single agar layer agar (SAL agar), and tryptone top agar were prepared as described in EPA method 1602.

Virus reduction test: 200 ml of a test water were forced through each of two identical units of the Sawyer 7/6BV filter (Fig. 2). Air pressure was regulated with a NorLab air regulator (model HPS270-125-590-4f, Norco Inc. Boise, ID). A 2 L Nalgene heavy-duty vacuum bottle (model DS2126, Nalge Nunc, Rochester, NY) was used as a pressure chamber. Water was forced through the filters at 40 psi.

Determination of efficacy: 100 ml of filtered water contained in a 250 ml Erlenmeyer flask were heated in a 44.5 °C water bath for three minutes. Then 5 ml of host bacteria

suspension, 5 ml calcium chloride solution, and 100 ml of SAL agar (also held at 44.5 °C) were added. The mixture was swirled and then poured evenly into 8 large perti plates (150 mm diameter).

Quality control: 1 ml of a 10^{-5} dilution of the test water added to 99 ml of sterile creek water used as a positive control and for enumeration of the test water. 100 ml of sterile creek water, not amended with phage, was passed through the test apparatus without a filter attached for use as a negative control. One unit of a commercially available carbon based water filter that does not make a viral reduction claim was used as an additional control.

Results and Conclusions

Test results: Table 2 displays the total numbers of plaques that were detected in 100 ml of test water passed through the filter units. No plaques were detected in the negative control. Thirty-nine plaques, corresponding to a pfu density of 3.9×10^6/100 ml were detected in the positive control sample. As such, the Sawyer 7/6BV filter reduced the virus concentration by 99.9997% (5.53 log units). The two units that were tested exceed the minimum requirements set forth in the Standard for removal of viruses by 1.53 orders of magnitude.

Table 2. Results of virus removal test for the Sawyer 7/6BV filter.

	Plaques (pfu/100mL)
Sawyer 7/6B unit 1	12
Sawyer 7/6B unit 2	11
Charcoal filter	3100
Negative control	0
Positiver control	3.9×10^6