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This is to certify that the Department of Soil, Water and Environmental Science of University of Arizona has evaluated the Puralytics SolarBag as per *U.S. EPA Guide Standard and Protocol for Evaluation of Microbiological Water Purifiers* and have found that the SolarBag exceeded the required reductions of bacteria, virus and protozoa.

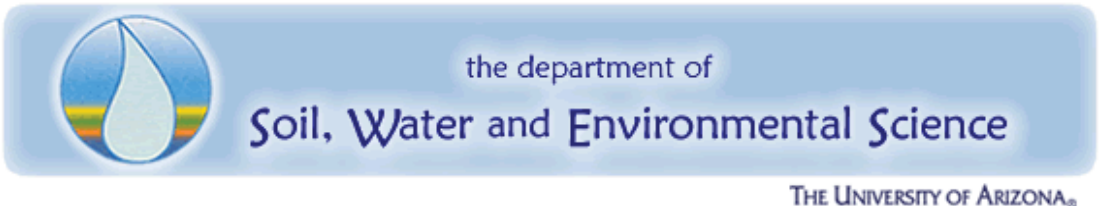
Puralytics may use the test report and results for marketing purposes and as independent test results of the SolarBag performance.

Puralytics may reference that the testing was performed at the University of Arizona.

Sincerely,

Charles P. Gerba, Ph.D.

Laura Y. Sifuentes, M.P.H.



Evaluation of Puralytics SolarBags for Removal of Bacteria, Virus and Protozoa According to the U.S. Environmental Protection Guide Standard and Protocol for Testing of Microbiological Purifiers

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Introduction

To ensure the efficacy of microbiological water purifiers the U.S. Environmental Protection Agency developed the Guide Standard and Protocol for Testing of Microbiological Water Purifiers, which was published in the Federal Register of May 26, 1986. This document provides the details for the test and performance requirements for devices designed to remove microorganisms from water. The guide establishes that any microbiological water purifier be capable of removing or killing enteric bacteria, viruses and protozoan parasites. Such units should be capable of reducing challenge levels of suggested microbial contaminates in each class of microorganism. The units must demonstrate at least a 99.9999% (6 log) removal of the enteric bacterium *Raoultella terrigena* (formally *Klebsiella terrigena*), a 99.99% removal of poliovirus and rotavirus, and a 99.9% removal of *Giardia*. *Cryptosporidium* has been substituted for *Giardia* because of its greater resistance to removal by disinfectants and filtration (Korich et al, 1990).

The purpose of this study was to assess the performance of Puralytics SolarBags to remove test microorganisms in accordance with the EPA Guide Standard and Protocol for Microbiological Water Purifiers.

Material and Methods

Six Puralytics SolarBags were supplied by the manufacturer (Puralytics, 15250 NW Greenbrier Parkway Beaverton, Oregon 97006-5764) and operated according to the manufacturer's instructions.

The units were challenged with both "General Case" (Test Water #1) and "Challenge Test Water "(Test Water # 2 – also referred to as "Worst Case"). Dechlorinated tap water from the University of Arizona (activated-carbon filtered) was used for the general case test water. The chemical/physical properties of this test water are show in Table 1. For the Challenge Test Water the dechlorinated tap water was used and the desired turbidity of the water adjusted by addition of approximately 88 mg/L of AC fine dust to obtain a turbidity of 30 NTU (GM, Flint, MI). total organic carbon (TOC) (10 mg/L was obtained by addition of approximately 23 mg/L of humic acid (Aldrich Chemical Company, WI), and Total Dissolved Solids (TDS) (1,200 mg/L added to obtain a final concentration of approximately 1,500 mg/L), by addition of 1.5 g/L of sea salts (Sigma Chemical Company, St. Louis, MO). The pH was adjusted to 9.0 by addition of 1 N NaOH). For the worst case water challenges the water was held in a refrigerator until the temperature reached 4 ^{o}C.

Table 1. Physical/chemical properties of Tapwater at the University of Arizona (General Case Test Water – Test Water #1)

pH	7.5-7.8
Total Organic Carbon	<1.0 mg/L
Turbidity	<1.0 NTU
Temperature	23-25 ^{o}C
Total Dissolved Solids	200-300 mg/L

Bacterial Analysis

R. terrigena (ATCC-33254) was grown overnight in Trypticase soy broth (EMD, Gibbstown, NJ) at 35 ^{o}C to obtain the organisms in the stationary growth phase. The bacterial cells were pelleted by centrifugation and resuspended in 0.25 M phosphate buffered saline at pH 7.0. This procedure was repeated three times to remove organic matter present in the broth. Bacterial assays were conducted using the spread plate method on EMB agar (EMD, Gibbstown, NJ).

Virus Analysis

Poliovirus type 1 (strain LSC-2ab), obtained from the Dept. of Virology and Epidemiology, Baylor College of Medicine, Houston TX and simian rotavirus (ATCC-VR-899) obtained from the American Type Culture Collection were used. Poliovirus was grown in the BGM cell line and rotavirus in the MA-104 cell line. After observation of extensive cytopathic effects, virus infected cells were harvested by three cycles of freeze-thawing of the infected cell monolayer. The cell lysates were then treated with an equal volume of Vertrel KF (Micro Care Corp., New Britain, CT) at 4 ^{o}C, stirred for 10 min on a magnetic stirrer and centrifuges at 10,000 rpm in a Beckman J2-21 centrifuge. The aqueous phase was collected and filtered through a sterile 0.45 µm pore-size membrane filter and stored at -70 ^{o}C till needed.

Titers of poliovirus and rotavirus were determined by the plaque forming unit method using 25 sq cm tissue culture flasks and 6 well cell culture plates (Smith and Gerba, 1982).

Cryptosporidium

Cryptosporidium parvum oocysts were obtained from the laboratory of Dr. Charles Sterling, University of Arizona. They were collected from the feces of infected calves and purified by discontinuous sucrose gradient (Arrowood et al. 1987). Infectivity of the oocysts was determined by the methods described in Di Giovanni et al. (1999) using infectivity in cell culture.

Test procedures

Worst case water was placed in 4 L beakers and stirred until reagents were completely dissolved. Test microorganisms were added and stirred for one minute (10^6 CFU/L bacteria, 10^6 PFU/L for viruses and 2 X 10^8 for *C. parvum*).

Three Puralytics bags each were filled with 2.5 liters of general case water or worst case water. The worst water was pre-filtered through pre-filter cloth (sock filters, with the elastic band at the top and attached to the cap lanyard). The material used in those filters is Pellon® - 40 wt.). The pre-filters were design to reduce the turbidity, but also were found to reduce the concentration of oocysts.

The bags were placed under direct sunlight for the duration of each experiment. Bags were placed on a cardboard surface with the labels facing down as instructed. The temperature of the water in the bag as well as the air temperature and air humidity was measured at each sampling time. In addition, the UV light intensity was also measured.

Samples were collected from the bags right after addition to the bags and after four hours exposure to sunlight. The organisms were tested on different days as indicated:

- 7/8/11-Poiovirus and rotavirus
- 7/9/11-*R. terrigena*
- 9/20/11-*Cryptosporidium parvum*

Table 2. Water and air temperatures, relative humidity and UV light intensity during testing

		Viruses		*R. terrigena*		*Cryptosporidium*	
Water Temps [^{o}C]		**7/8/2011**		**7/9/2011**		**9/20/2011**	
	Exposure Time (Hours)	0	4	0	4	0	4
General Case Water	**Replicate 1**	25	48	24.3	54.7	27.7	56
	Replicate 2	25.8	48	24.7	54	27.6	55.5
	Replicate 3	26	48	25	53.4	27.9	56.9
Worse Case Water	**Replicate 1**	25.4	48.4	26	53.4	28.5	60.2
	Replicate 2	25.7	47.9	25.3	54	27.7	59.9
	Replicate 3	25.4	47.9	24.2	53.6	27.8	60.7
Outdoor Temps [^{o}C]		31.8	41	28.4	43	35.3	36.6
Relative Humidity [%]		53	24	47	38.5	46	20
UV 280-400 nm [mW/cm^{2}]		31.4	27.8	24	19.5	30	35.2

Table 3 shows the reductions in poliovirus type 1 and rotavirus SA-11 within the SolarBags. Both viruses were reduced below detection or by greater than 99.9999% or 6 logs. The test bacterium was also reduced by greater than 6 logs (Table 4). *Cryptosporidium parvum* was reduced by almost 2 logs by filtration and another two logs by the sunlight exposure achieving from 3.3 to 4.10 log removals (Table 5).

Table 3: Log Reduction of Rotavirus and Poliovirus after filtration and 4 hour exposure to sunlight.

	Worse Case Water			General Water		
Virus	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Replicate 1**	**Replicate 2**	**Replicate 3**
Poliovirus (PV-1)	> 6	> 6	> 6	> 6	> 6	> 6
Rotavirus (SA-11)	> 6	> 6	> 6	> 6	> 6	> 6

Table 4. Log Reduction of *R. terrigena* after filtration and 4 hour exposure to sunlight.

	Worse Case Water			General Water		
Bacteria	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Replicate 1**	**Replicate 2**	**Replicate 3**
R. terrigena	> 6	> 6	> 6	> 6	> 6	> 6

Table 5: Log reduction inactivation of *C. parvum* after filtration and 4 hour exposure to sunlight.

	Worse Case Water			General Water		
Time (Hour)	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Replicate 1**	**Replicate 2**	**Replicate 3**
0	1.72*	1.70	1.82	1.72	1.7	1.7
4	3.52	4.10	3.36	3.94	3.81	3.42

*loss in oocysts due to filtration

Conclusions

The Puralytics SolarBags exceeded the required reductions of the test organisms as required for microbiological water purifiers after a four hour exposure to sunlight.

References

Arrowood, M.J. et al. 1987. Isolation of oocysts and sporozoites using a discontinuous sucrose and isopynic gradients. J. Parasitology 73:314-319.

Di Giovanni, G. D. et al. 1999. Detection of infectious *Cryptosporidium parvum* oocysts in surface and filter backwash water samples by immunomagnetic separation and integrated cell culture-PCR. Appl. Environ. Microbiol. 65: 3427-3432.

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