VESTERGAARD FRANDSEN

DISEASE CONTROL TEXTILES

Working Principle of LifeStraw®

At the time of use, the bottom and mouthpiece caps are to be opened. Water is drawn up through the mouthpiece (LifeStraw® is used like a regular straw, see pictures).

The plastic prefilter located at the bottom of the LifeStraw® removes coarse particles, larger than 1mm, from the water.

The vacuum applied during the sucking process forces the water to pass through the 0.2microns porosity hollow-fibre microfiltration membrane.

The purification process happens through the microfiltration membrane which stops all particles larger than 0.2µm (including protozoan parasites and bacteria). Turbidity particles are also stopped by the membrane by size exclusion. The untreated water is pushed through the ultrafiltration (hollow-fibre) membrane by the vacuum applied during the sucking process, particles and microbes larger than 0.2µm stay on the dirty side of the membrane and clean water passes through the membrane. Filtered water is ingested from the mouthpiece.

The LifeStraw® is cleaned by blowing air through the mouthpiece. When performing the cleaning step, dirt particles located on the dirty side of the membrane are lifted by backpressure and then removed by flushing through the bottom opening.

Since all bacteria and protozoan parasites are stopped by the 0.2µm membrane, the filtered water complies with the USEPA requirements of LOG 6 and 3 reductions for water filters for these two groups of microbes.

The vacuum which allows the filtration process leads to a flow-rate of around 280 mL/min (200mL/min in average during the lifetime of the filter).

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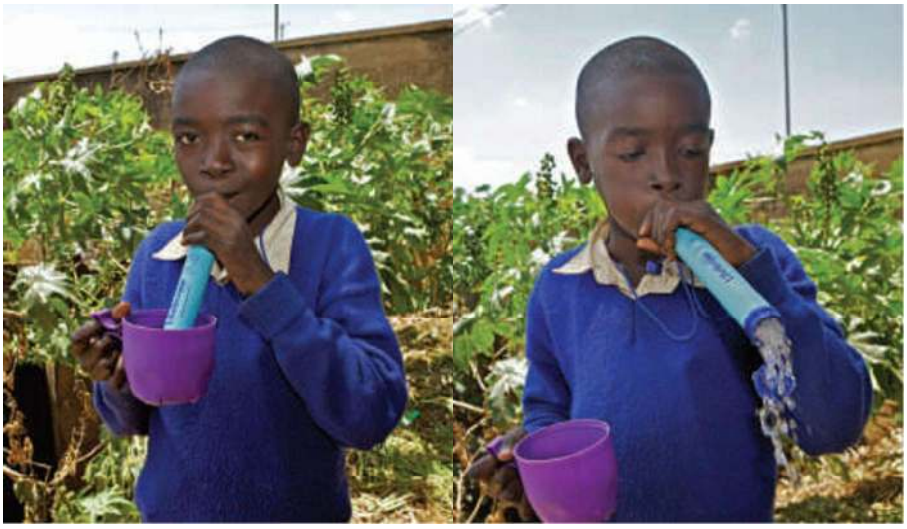

1) Place LifeStraw® in water and sip through the mouthpiece.

2) Regularly blow through LifeStraw® after drinking to keep the filters clean and to prevent them from clogging

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LifeStraw

Efficacy Data

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This is to certify that Department of Soil, Water and Environmental Science of University of Arizona have evaluated LifeStraw–Hollow Fiber filter as per U.S. EPA guide standard and protocol for evaluation of microbiological water purifiers and have found following satisfactory results:

- Turbidity removal during the challenges by 99.6% in average.
- The unit achieved the required geometric average removal of 7.3 $\log_{10}$ for *Escherichia coli* (which is higher than the required EPA standard of 6 $\log_{10}$) and 3.9 log 10 for *Cryptosporidium* oocysts at all test points (higher than the USEPA requirement of 3 $\log_{10}$)
- The units performed well when challenged with "worst case" water quality.

Vestergaard-Frandsen may use the above claims or suitable modifications of the above claims for its marketing/PR/website/regulatory purposes.

Vestergaard-Frandsen may say that the work was performed at the University of Arizona in any public material with respect to the claims mentioned/modified as above.

Sincerely,

Charles P. Gerba
Professor

Jaime E. Naranjo
Research Specialist, Principal

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EVALUATION OF VESTERGARD-FRANDSEN'S HOLLOW FIBER LifeStraw® FOR THE REMOVALOF ESCHERICHIA COLI AND CRYPTOSPORIDIUM ACCORDING TO THE U.S. ENVIRONMENTAL PROTECTION AGENCY GUIDE STANDARD AND PROTOCOL FOR EVALUATION OF MICROBIOLOGICAL WATER PURIFIERS

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and

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Department of Soil, Water and
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May 17, 2010

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SUMMARY

Three identical Vestergaard-Frandsen's hollow fiber LifeStraw were evaluated for their ability to remove *Escherichia coli*, and *Cryptosporidium* oocysts. The units were operated according to the manufactures instructions until 1625 liters had been processed. The units were challenged with the test microorganisms after 0,250, 500, 600, 750, 900, 1000, 1150, 1250, 1450, 1500, 1575 and 1625 liters had passed through the units. Aging water used for the evaluation of the units had a turbidity of 15 NTU, total organic carbon of 5 mg/L (humic acid) and 400 mg/l total dissolved solids. All challenge tests were conducted with "worst case" water quality of 1500 mg/l dissolved solids, 10 mg/l organic matter, +25°C, with a turbidity of 100 NTU and a pH of 7.8. And a cleaning procedure of backwashing the filter every five liters with air was also performed. The geometric average removals exceeded 99.9999% for the bacteria, and 99.9% for the *Cryptosporidium* oocysts. These units comply with the criteria guidelines under the U.S. Environmental Protection Agency's Guide Standard and Protocol for Testing Microbiological Water Purifiers for these two groups of microbes.

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INTRODUCTION

Development of the need for personal water treatment devices has evolved from consumer interest in improving and ensuring the quality of drinking water. The need also extends to the quality of untreated or partially treated waters such as that used by hikers, campers, recreational home and boat owners.

One of the major concerns in water treatment is the need to remove disease-causing microorganisms (bacteria, and protozoa) from water before its consumption, since it is recognized that infectious disease transmission by water is a significant public health concern. The majority of documented waterborne diseases in the United States are still caused by infectious microorganisms (Craun, 1986).

It is important that water treatment units or devices designed for the protection of human health be effective against pathogenic microorganisms and be capable of providing this capability over the life of the equipment and over a wide range of water conditions. This requirement is a necessary consideration for protection of the public's health by both the water industry and the government.

To ensure the efficacy of microbiological water purifiers, a multidisciplinary task force was formed by the U.S. Environmental Protection Agency to develop a guide standard and protocol for testing of such units. This guide standard and protocol appeared in the Federal Register of May 26, 1986, and has been accepted on a provisional basis by the U.S. Environmental Protection Agency's Office of Drinking Water and Office of Pesticide Programs. This document recommends test and

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performance requirements for microbiological water purifiers. While the document specifically deals with testing criteria for certain types of water treatment devices such as halogen disinfects, ultraviolet light, ceramic filters, etc., its purpose was to serve as a guide for all types of water treatment devices. The guide establishes that any microbiological water purifier be capable of removing or killing enteric bacteria 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% removal of Escherichia coli, and a 99.9% removal of *Giardia*. The devices must also be capable of achieving these results under a realistic "worst case" water quality situation.

To assess the performance of the units, *Giardia* cysts were replaced with *Cryptosporidium* oocysts as the test parasite. *Cryptosporidium* oocysts (3.0-5µm) are smaller than *Giardia* cysts (8.0-12µm) and more likely to pass through units, which depend upon filtration for parasite removal. *Cryptosporidium* is extremely resistant to common water disinfectants (Korich et al, 1990) and has caused several large waterborne outbreaks in the United States and Europe in recent years (Smith and Rose, 1990). Thus, any filtration unit capable of removing *Cryptosporidium* should be able to eliminate *Giardia* cysts. It was recently recommended by the FIFRA Scientific Advisory Panel Antimicrobial Subpanel, Office of Pesticide Programs, on August 24, 1993, that *Cryptosporidium* oocysts be substituted for *Giardia* cysts for testing microbiological water purifiers.

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MATERIALS AND METHODS

Experimental Design

The basic experimental design for evaluating the water purification units was based on the recommendations of the U.S. Environmental Protection Agency's Task Force Report on the *Guide Standard and Protocol for Testing Microbiological Water Purifiers* (Federal Register, May 26, 1986).

Three hollow fiber LifeStraw were provided by Vestergaard-Frandsen, Ch. De Messidor, 5-7, CH-1006 Lausanne, Switzerland and operated according to the manufacturer's instructions. The units were challenged with the test microorganisms after various points of lifetime of operation. Between challenges dechlorinated (by passage of the tapwater through a column of activated carbon) University of Arizona tapwater was processed through the units and a backflushing procedure was done every five liters with the aid of a MIKASA double barrel pump (Irvine, CA). The physical/chemical characteristics of this water are shown in Table 1. The units were challenged with the microorganisms suspended in "worst case" water quality at all challenge points. For the worst case microbial challenge, the turbidity of the test water was increased to 100 NTU by addition of ISO 12103-1, A2 fine test dust (Powder Technology, inc., Burnsville, MN), 1500 mg/l dissolved solids by addition of sea salts (Sigma Chemical, St. Louis), and 10 mg/l humic acid (Aldrich, Milwaukee, WI).

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Bacterial Analysis

Escherichia coli (ATCC-25922)) was grown overnight in Trypticase Soy broth (Difco, Detroit, MI) at 37°C to obtain the organisms in the stationary growth phase. The bacterial cells were pelleted by centrifugation and resuspended in phosphate buffered saline. This procedure was repeated three times to remove organic matter present in the broth.

Bacterial assays were conducted by the membrane filtration method on m-Endo Agar LES (Becton Dickinson and Cockeysville, MD. Cat# 4311203). Appropriate dilutions of influent samples were made in sterile 0.025 M phosphate buffered saline (PBS) at pH 7.0. A 100 mL sample of undiluted unit effluent was also assayed.

Cryptosporidium Assay

Cryptosporidium oocysts were obtained from feces of infected calves and purified by a discontinuous sucrose gradient procedure (Arrowood and Sterling, 1987). Unit influent (10 mL) and effluent (100 mL) were collected separately. They were centrifuged in an IEC Clinical Centrifuge (Nedhan Hts, MA) at 400 x g for 15 minutes to pellet the oocysts. The supernatant was aspirated to one mL above the pellet. After resuspension of the pellet in phosphate base buffer, the oocysts were counted using a Spotlite hemocytometer (Baxter Healthcare Corp. McGraw Park, IL) using a phase microscopy (BH Olympus, Japan) at 400x magnification. At least 12 chamber aliquots were counted for each sample according to the procedure outlined in the Guidance Manual (USEPA, 1990). The average of the total counts of oocysts were divide it by 9

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(number of squares counted under each chamber of hemocytometer) and this number was multiplied by $1.0x10^4$ to obtain the number of oocysts per mL in the concentrate. The total number of oocysts was divided by 10 in the case of influent samples and by 100 for effluent to determine the number of oocysts per mL of the test water before and after passage through the unit.

RESULTS

The results of microbial removals are shown in Tables 2 through 4. These results show that the units achieved the required geometric average removal of 7.3 $\log_{10}$ for *Escherichia coli* (USEPA requires 6 $\log_{10}$), and 3.9 $\log_{10}$ for *Cryptosporidium* oocysts at all test points (USEPA requires 3 $\log_{10}$).

Turbidity was removed during the challenges by 99.6% in average.

Flow-rates varied as follows in average:

- 280mL/min at the beginning
- 280mL/min between 10 and 200L
- 250mL/min between 200 and 500L
- 170mL/min between 500 and 1000L
- 111mL/min between 1000 and 1525L

Between 0 and 1000L the average flow-rate was 200mL/min

In summary, the Vestergaard-Frandsen hollow fiber LifeStraw met the microbial removal requirement of the U.S. Environmental Protection Agency's *Guide Standard and Protocol for Testing Microbiological Water Purifiers* for bacteria and parasites.

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Table 1. Test Waters Used in Microbiological Challenges

Aging Test Water 25 °C	
Turbidity (NTU)	15
pH	7.8
Total Dissolved Solids (mg/l)	450
Total Organic Carbon (mg/l)	5
"Worse Case" Challenge Water 25 °C	
Turbidity (NTU)	100
pH	7.8
Total Dissolved Solids (mg/l)	1500
Total Organic Carbon (mg/l)	10

Table 2. Removal of *Escherichia coli*
Results are given as colony forming unit per liter

CHALLENGE POINT	LITERS	INFLUENT	UNIT 1 EFFLUENT	UNIT 2 EFFLUENT	UNIT 3 EFFLUENT	LOG REDUCTION	PERCENT REDUCTION
0	0	1.69E8	<10	<10	<10	>7.23	>99.999995
15	250	1.20E8	<10	<10	<10	>7.08	>99.999992
30	500	2.00E8	<10	<10	<10	>7.30	>99.999995
37	600	1.45e8	<10	<10	<10	>7.16	>99.999993
46	750	2.50e8	<10	<10	<10	>7.40	>99.999996
55	900	1.02e8	<10	<10	<10	>7.01	>99.99999
61	1000	1.40e8	<10	<10	<10	>7.15	>99.999993
70	1150	2.70e8	<10	<10	<10	>7.43	>99.999996
76	1250	3.50e8	<10	<10	<10	>7.54	>99.999997
89	1450	2.10e8	<10	<10	<10	>7.32	>99.999995
92	1500	1.56e8	<10	<10	<10	>7.19	>99.999993
96	1575	5.00e8	<10	<10	<10	>7.70	>99.999998
100	1625	7.00e8	<10	<10	<10	>7.84	>99.999998

Table 3.Removal of *Cryptosporidium* oocysts
Results are given as oocysts per liter

CHALLENGE POINT	LITERS	INFLUENT	UNIT 1 EFFLUENT	UNIT 2 EFFLUENT	UNIT 3 EFFLUENT	LOG REDUCTION	PERCENT REDUCTION
0	0	5.42e6	6.94e2	<6.94e2	<6.94e2	>3.89	>99.98
15	250	6.25e6	<6.94e2	<6.94e2	<6.94e2	>3.95	>99.98
30	500	5.00e6	<6.94e2	<6.94e2	<6.94e2	>3.86	>99.98
37	600	5.75e6	<6.94e2	<6.94e2	<6.94e2	>3.92	>99.98
46	750	6.58e6	<6.94e2	<6.94e2	<6.94e2	>3.98	>99.98
55	900	5.91e6	<6.94e2	<6.94e2	<6.94e2	>3.93	>99.98
61	1000	5.58e6	<6.94e2	<6.94e2	<6.94e2	>3.91	>99.98
70	1150	6.00e6	<6.94e2	<6.94e2	<6.94e2	>3.94	>99.98
76	1250	5.54e6	<6.94e2	<6.94e2	<6.94e2	>3.90	>99.98
89	1450	5.60e6	<6.94e2	<6.94e2	<6.94e2	>3.91	>99.98
92	1500	6.10e6	<6.94e2	<6.94e2	<6.94e2	>3.94	>99.98
96	1575	5.80e6	<6.94e2	<6.94e2	<6.94e2	>3.92	>99.98
100	1625	6.90e6	<6.94e2	<6.94e2	<6.94e2	>3.99	>99.98

Table 4 Over All Percent and Log Reduction

Microorganism	Log Reduction	Percent Reduction
Escherichia coli	>7.33	>99.999995
Cryptosporidium oocysts	>3.93	>99.98

Table 5. Flow Rate
Milliliters per minute
Cleaning Procedure every 5 liters

Challenge points in liters	Flow Rate
0	280
10 to 100	280
200 to 500	250
500 to 1000	170
1000 to 1525	111

Table 6. Average Turbidity Removal.
Results are given as NTU

Influent	104
Effluent	0.4