Institut Pasteur de Lille

TEST REPORT

REMOVAL OF MICROORGANISMS FROM WATER BY GEYSER FILTERS ARAGON-BIO

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1. OBJECTIVE

GEYSER filters ARAGON-BIO are designed for purification of water at home. The aim of this study was to evaluate the efficiency of these filters to remove microbial contamination from water. Institut Pasteur de Lille was contacted to perform these tests using bacteria and viruses that are likely to contaminate water networks. Comparison of microbial concentration in water before and after filtration was performed using artificially contaminated ultrapure water.

2. MATERIAL AND METHODS

2.1. STRAINS AND MEDIA

Test strains were chosen to be representative of contaminants that may be found in water networks. They include 2 bacteria and 3 viruses:

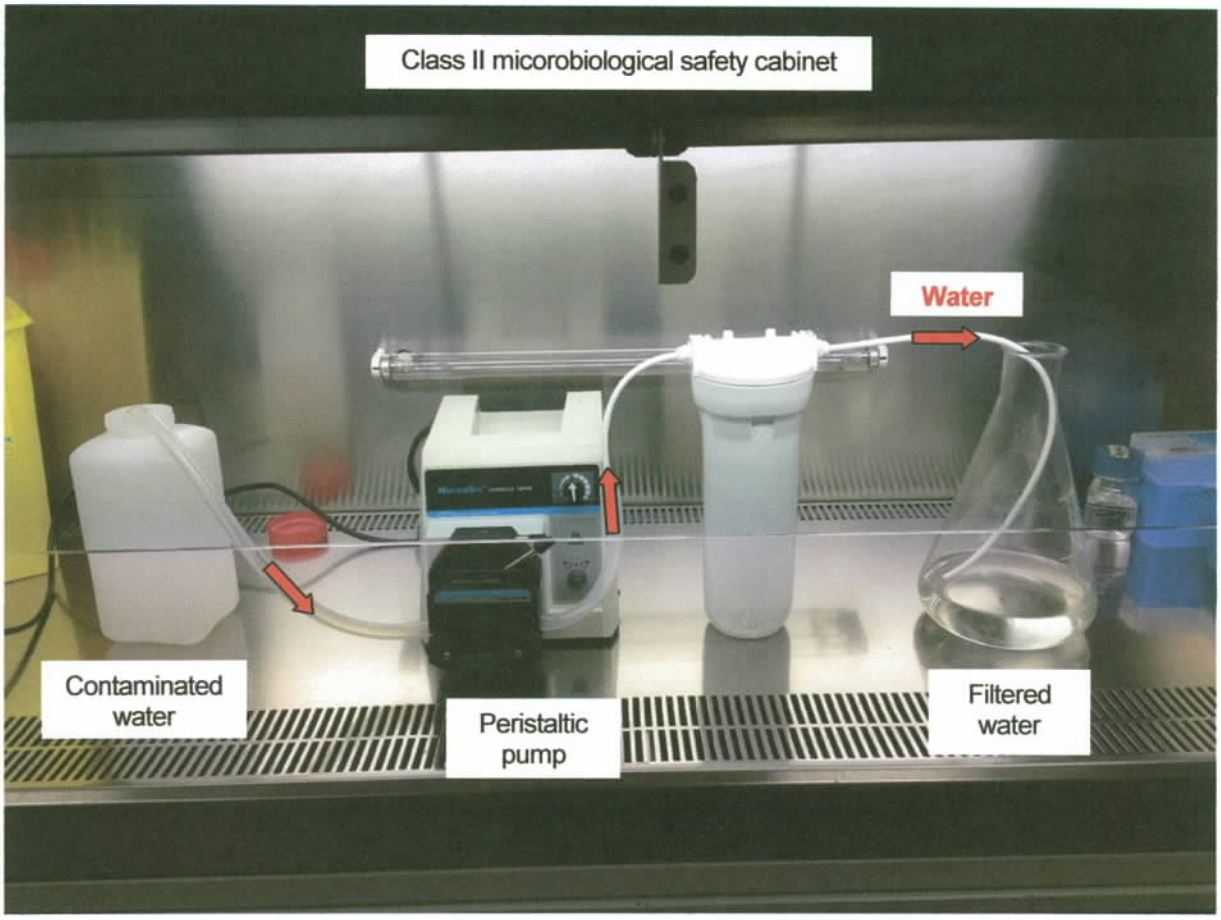
- *Salmonella* Typhimurium (ATCC 14028); growth and enumeration were performed on Trypticase Soy Agar medium.
- *Legionella pneumophila* serogroup 1 (CIP 103854); growth and enumeration were performed on Buffered Charcoal-Yeast Extract agar medium.
- Poliovirus, strain Sabin type 1; production and titration were performed of BGMK cells (African Green Monkey kidney cells).
- Rotavirus, strain simiens SA114F1; production and titration were performed on MA104 cells (ATCC CRL-2378).
- Hepatitis A virus (HAV), strain HM175/18f (ATCC VR-1402); production and titration were performed on FRhK4 cells (ATCC CRL-1688).

Bacteria were counted in Colony-Forming Units (CFU). Viral titers for Poliovirus and Rotavirus were calculated using the Most Probable Number method, as described in the French Standard XP T90-451, and expressed in Cytopathic Units (CU). HAV titers were counted in Plaque-Forming Units (PFU).

2.2. EXPERIMENTAL SETTING

A peristaltic pump was used to circulate water through the filters at 0.8 L/min. Ultrapure water obtained from an arium® system (Sartorius AG, Germany) was used throughout the study. Each filter was used once.

Since test strains are pathogenic microorganisms, all experiments were conducted inside a class II microbiological safety cabinet, in a BSL 2 laboratory.



Prior to each experiment, 5 L of non-contaminated ultrapure water were pumped through the filter. The following procedure was then used for all experiments:

- A contaminating solution was prepared, which contained approximately 10^8 CFU/mL (bacteria) or 10^8 CU/mL (viruses), and was enumerated;
- 1.3 L of ultrapure water was contaminated using 1 mL of the contaminating solution;
- Contaminated water was circulated through the filter using the peristaltic pump at 0.8 L/min, with filtered water being collected from the output of the filter;
- Immediately after the contaminated water, 0.7 L of non-contaminated ultrapure water was pumped through the filter, after which the pump was left working for 1 minute, with all filtered water collected;
- The total volume of collected filtered water was measured, and it was used to evaluate the quantity of microorganisms remaining after filtration.

The quantity of bacteria in filtered water was evaluated by filtering it through a 0.45 µm membrane, which was then placed on agar growth medium. Five membranes were used for each experiment, with the following volumes filtered: 0.1 mL, 1 mL, 10 mL, 100 mL, and the remaining volume of water (close to 2 L). Since the total volume was filtered on membranes, it was theoretically possible to detect even 1 CFU in the whole volume.

For viruses, a sample of filtered water was used to evaluate the viral concentration by titration. In this case, it was not possible to test the whole volume. The theoretical limit of detection was about 1 CU/mL, corresponding roughly to 2×10^3 CU in the whole volume.

Using the initial quantity of microorganisms in water (Q_{in}) and the quantity left in filtered water (Q_{out}), the filtration efficiency was evaluated by calculating the amount of microorganisms that were removed from water by the filter, as follows:

$$\% \text{ removed} = (Q_{in} - Q_{out}) / Q_{in}$$

or equivalently

$$\text{Log removal} = \text{Log}_{10}(Q_{in}) - \text{Log}_{10}(Q_{out})$$

Q_{in} and Q_{out} are expressed in CFU (bacteria), PFU (HAV), or CU (other viruses), and were evaluated using the microbial concentration in a given solution (C) and the volume of that solution (V), as

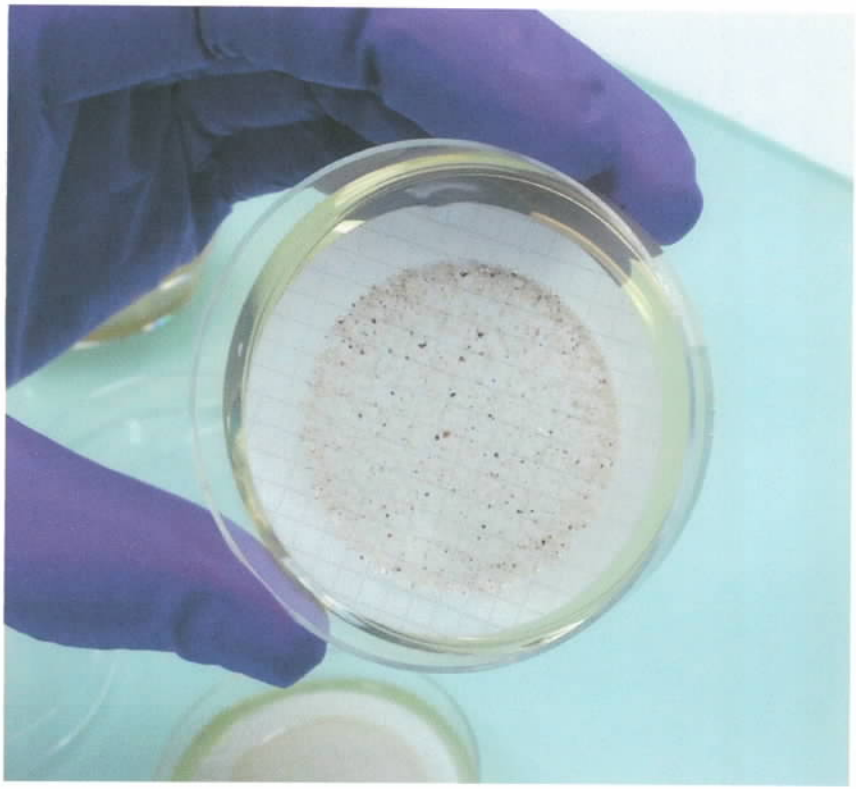
$$Q = C \times V$$

Each experiment was repeated, with a total of 3 runs for each condition.

3. RESULTS

Each cartridge was opened prior to the experiment to check the reference number written at the bottom of the filter. All tested filters but one had the same reference number: **25763**. The only exception was the filter used for the third test on poliovirus, for which the reference number was **25763 2**.

It appears that material from the filter could be found in filtered water. This was particularly noticeable when passing water through a membrane for experiments with bacteria: the picture below shows the membrane through which 2.018 L had been filtered, for the first run on *Salmonella* (this membrane was the one with greatest load of filter material).



Results are presented in the table below.

Strain	Run	Collected volume (L)	Q_{in} (CFU, CU, or PFU)	Q_{out} (CFU, CU, or PFU)	% removed	Log removal	Mean Log removal
Legionella	1	2.134	$5.4x10^7$	$1.5x10^2$	99.9997%	5.6	5.9
Legionella	2	2.108	$5.4x10^7$	$2.9x10^1$	99.99995%	6.3	
Legionella	3	2.084	$5.4x10^7$	$8.2x10^1$	99.9998%	5.8	
Salmonella	1	2.130	$1.9x10^8$	$3.2x10^6$	98.3%	1.8	4.8
Salmonella	2	2.047	$1.9x10^8$	$1.1x10^3$	99.9994%	5.3	
Salmonella	3	2.186	$2.7x10^8$	$1.5x10^1$	99.999994%	7.3	
Poliovirus	1	2.255	$1.2x10^8$	$1.6x10^5$	99.86%	2.9	2.8
Poliovirus	2	2.165	$1.2x10^8$	$2.0x10^5$	99.83%	2.8	
Poliovirus	3	2.085	$1.1x10^8$	$2.3x10^5$	99.79%	2.7	
Rotavirus	1	2.080	$3.6x10^8$	$5.1x10^4$	99.986%	3.9	4.0
Rotavirus	2	2.000	$3.6x10^8$	$2.6x10^4$	99.993%	4.1	
Rotavirus	3	2.018	$3.6x10^8$	$4.1x10^4$	99.989%	3.9	
HAV	1	2.130	$4.3x10^8$	$2.3x10^7$	93.4%	1.2	1.4
HAV	2	2.108	$4.3x10^8$	$3.3x10^7$	92.3%	1.1	
HAV	3	2.095	$8.5x10^8$	$1.3x10^7$	98.5%	1.8	

In the first run on *Salmonella* Typhimurium, the filtration efficiency was significantly lower than for the other trials. This unexpected result indicates that the filter used in this run was not able to remove bacteria from water. Whether the filter was broken or if the water tightness was otherwise compromised could not be verified, since filters were destroyed immediately after the experiments, whereas microbiological counts were obtained after one or several days of incubation.

4. CONCLUSION

Geyser filters ARAGON-BIO were able to remove the tested bacteria from water, with log reductions ranging from 5.3 to 7.3, with one exception for a run on *Salmonella* Typhimurium. Removal efficiency was lower for viruses, with mean log reductions of 2.8 for poliovirus, 4.0 for rotavirus, and 1.4 for hepatitis A virus.