Evaluation of *Cryptosporidium parvum* oocyst removal by an Aquamira water bottle filter

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The ability of an Aquamira water bottle filter unit to remove *Cryptosporidium parvum* oocysts was tested. Test water consisting of a suspension of *Cryptosporidium parvum* oocysts in stream water was squeezed through the filter in the same way as the unit is designed to be used. The test water was then collected and passed through an additional laboratory filtering apparatus to capture oocysts in the test water onto a film. The film was stained with a fluorescent DNA-binding dye and observed for oocysts under a fluorescent microscope. Unit efficiency was calculated based on comparison to a positive control. The unit removed greater than 99.99% of oocysts (Table 1). No oocysts were determined to have passed through the filter.

Table 1. Oocysts removal by an Aquamira water bottle filter unit.

	Oocysts (ave. mm^{-2})	Sphere removal (proportion)	Unit Efficiency (%)
Aquamira bottle filter	0.0	1.0000	>99.99
Positive control	237.2		
Negative control	0		

Method

Test water: Test water was prepared by adding live oocysts purchased in solution from Waterborne, Inc (New Orleans, LA) to 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.

Conditioning water: Autoclaved, room temperature, water from Rattlesnake Creek without added oocysts was used for conditioning water.

Oocyst reduction test: The unit was conditioned with one liter of water prior to testing. 100 mL of test water were squeezed through the filter unit. The first 50 mL of test water

were discarded to ensure that conditioning water was not included in the test sample. 50 mL were collected for counting.

Determination of efficacy: Protozoans were collected on 0.2 μm black filters (Osmonics 11021) and stained with 4',6'-diamidino-2-phenylindole hydrochloride (DAPI) for microscopic counting using a Zeiss epifluorescent microscope. For positive controls the average number of cells in twenty fields of view was used to calculate cell numbers. To determine the number of oocysts on a film the microscope was first focused on the film and examined. If several oocysts were seen in the first few fields of view then at least 10 fields of view were counted. If oocysts were not seen, then the microscope was passed more than 5 times across the entire film, representing several hundred fields of view. Oocysts are reported according to the number of oocysts detected per mm^2 of the laboratory film.

Quality control: Several different units were tested at the same time. Testing of one unit was replicated three times for quality control purposes. The volume of water trapped on the films was varied for the replicates to ensure accurate counting. 50 mL of filtered test water were trapped onto the films for two replicates. For the third replicate, 40 mL of test water was trapped on the film. The quality control procedure indicated that counts were accurate (see report AQ06-03.5).