Evaluation of a Tangential-Flow Multiple-Filter Technique for Detection of Giardia lamblia Cysts in Water

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A system of tangential-flow filtration was evaluated for use in the detection of Giardia cysts in drinking water. This method was more sensitive in recovering cysts than a frequently used wound-orlon system of through-filtration.

Giardia lamblia is the most frequently identified agent of waterborne disease in the United States (2). Many aspects of this parasitic infection make the development of sensitive and specific methods for detecting the infective cyst stage difficult. The method of Swed by culture of the vegetative trophozoite stage is not routinely available and since elevated coliform counts are not a reliable indication of the presence of Giardia cysts (3), laboratory procedures providing evidence of giardia-contaminated drinking water are restricted to techniques to concentrate the water sample, followed by microscopy to identify the parasite. Also, low concentrations of cysts may result in waterborne disease (5), although their presence would be difficult to demonstrate in water by using currently available laboratory techniques.

We evaluated the Millipore Pellicon cassette system (Millipore Corp., Bedford, Mass.) of concentrating water as a method with potential advantages for demonstrating the presence of Giardia cysts in drinking water.

The Pellicon cassette system consists of a high-volume cell (filter holder) which manifolds incoming fluids, distributing them in tangential-flow paths across parallel layers of membrane (Fig. 1). A stack of 5 ft2 (~0.4645 m2) of microporous filters (Durapore, HVLP, 0.5 μm) was used in these experiments. G. lamblia cysts were obtained from fresh feces of infected patients who submitted specimens to the Provincial Laboratories, British Columbia, Canada. Cysts were harvested by using a sucrose gradient method of concentration (1). Stocks of harvested cysts were suspended in water and kept for a maximum of 3 weeks at 4°C. A standard inoculum was established by appropriate dilutions of each stock according to a count determined by using the hemacytometer method. Stocks from different patient sources were tested separately and were considered to be different strains of Giardia.

The quantitation of cysts by either direct microscopic counts or hemacytometer (Neubauer chamber) counts was found to be unreliable for the purpose of accurate evaluation of cyst recovery by using our inoculum of relatively low cyst concentration. The method of Spaulding et al. (7) was used to quantitate cysts in each control and test sample. Inocula were prepared by mixing the appropriate volume of stock suspension to achieve a final count of approximately 102 to 103 cysts. The cysts were suspended in 100 ml of distilled water, i.e., 1 to 10 cysts per ml, for ease of thorough mixing. For each run of test samples, one of these samples (the control) was passed directly through a 1.2-μm membrane filter (Millipore; 25 mm, RAWG grid) for counting. Test samples of cysts were inoculated into 4.0 liters of premixed distilled water and then fed into the membrane system by using a low-shear peristaltic pump. By using the recirculation mode, suspensions were separated by this system into a filtrate and a concentrated solution (retentate) containing the Giardia cysts. The retentate volume was reduced to 250 ml by recirculation, and this cyst suspension was then passed through the 1.2-μm filter system for counting. The 1.2-μm (25 mm) filter was fixed, stained, and examined by microscopy by using the same method that was used for unprocessed controls. Cyst recovery was calculated by comparing the number of cysts on the stained preparation of processed suspension with that of the control.

The microporous membrane stack was flushed with large volumes of distilled water, and at the end of each run, it was cleaned with 1 N sodium hydroxide and stored in 70% ethanol as recommended by the manufacturer.

Six different strains of G. lamblia were tested. Cysts were harvested from the feces of six persons who submitted unpreserved specimens. A minimum of three tests, and for most strains more than three tests, were carried out for each strain. A minimum of one control was used for each run. When more than one control sample was processed, an average of the counts obtained was calculated. Results are found in Table 1.

The overall recovery of inoculated cysts was 31%. Recovery rates for four of the six strains tested were similar. To explain the low recovery rate of two strains, we examined the age of each strain tested but found no correlation between the age of cysts and decreased recovery after concentration.

Other investigators noted that the method of filtration recommended by the Environmental Protection Agency (EPA) has yielded recovery rates of 5 to 15% (4). To rule out possible strain differences, we quantitatively compared this method with results obtained by using another local Giardia strain and the present procedure. We conducted three experiments, following a standard procedure (EPA method) and counting cysts by using the method of Spaulding et al. (7), which yielded a <1% cyst recovery. A test and control specimen of the same strain in the Pellicon system yielded a recovery rate of 16%.

To establish where the loss of Giardia cysts occurred in the Pellicon system, we examined the sediment of distilled water with 10% buffered citrate-polyborate detergent, which was centrifuged after it was used to wash various components of the system, including the membrane (before

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cleaning) and the 5-liter flask that was used to deliver the cyst suspension into the test system. Cysts were identified in the sediment from postrun detergent washes of the membranes, the silicon tubing, and the 5-liter flask. Several cysts were found repeatedly in both the Schaudinn fixative and the alcohol used to stain the 1.2-μm (25 mm) filter.

The time to complete each test filtration was approximately 30 min, the fixing-staining time was 25 min, and the reading time by microscopy of each entire 1.2-μm (25 mm) filter was 3 to 4 h.

There is considerable interest in the further development of a reliable and practical method of detecting the infective cyst stage in drinking water. The most widely used procedure (EPA method) for detection of Giardia cysts in water involves filtering a 380-liter sample through a 25-cm-long, yarn-wound orlon filter (7 μm) housed in a plastic holder. In the laboratory, the filter cartridge is placed in a tray or pan, and the orlon fibers are unwound (or segmented), removed from the filter support, and washed. This wash from the segmented fibers is concentrated, and the sediment is examined by microscope for the presence of G. lamblia cysts.

When preliminary experiments with the Pellicon system of tangential-flow filtration showed good recovery of cysts concentrated from large volumes of tap water, a full assessment of the sensitivity of this method was undertaken. Since, in this system, particulate matter does not pass through the filter stack but is concentrated by being passed along a system of membranes, recovery of cysts from material other than what is present in the sample drinking water is not necessary. In our evaluation, we found that the system of Spaulding et al. (7) for counting cysts (passing concentrated samples through a small 1.2-μm disposable filter and fixing, staining, and examining the filter by microscope) was a more accurate microscopic method than either direct counts or the hemacytometer method.

Investigators examined the recovery rate of the EPA method (4) and found it to be about 5 to 15%. When compared with this orlon-wound EPA method, the present method was found to be more sensitive. The time-consuming procedure of microscopic examination necessary to identify the cysts is common to both methods, but we found the processing of individual cyst-containing orlon-wound filters (required in the EPA method before microscopy) both laborious and crude. This separation procedure is not necessary in the membrane system of tangential-flow filtration.

Clearly, field testing will be necessary to further evaluate the potential usefulness of this method of cyst recovery. Compared with the relatively small orlon-wound filter system, the Pellicon system is less portable and requires more expertise in concentrating the large-volume sample of water to be examined.

The use of monoclonal antibodies to detect G. lamblia cysts in water samples (6) was recently described. Combined with a more sensitive method of sample concentration and cyst recovery as demonstrated in this study, these other new techniques should improve the rate of laboratory confirmations when waterborne giardiasis is suspected.

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LITERATURE CITED


