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Reply to "Ranking Filter Methods for Concentrating Pathogens in Lake Water"

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Accurately comparing filtration methods is indeed difficult. Our method (1) and the method described by Borchardt et al. for determining recoveries are both acceptable approaches; however, each is designed to achieve a different research goal. Our study was designed to compare recoveries of multiple microorganisms in surface-water samples. Because, in practice, water-matrix effects come into play throughout filtration, concentration, and detection processes, we felt it important to incorporate those effects into the recovery results.

In our study, the concentrations of microorganisms were measured prior to seeding the test sample. The concentrations of the seed organisms were determined in the absence of any matrix effect from the water sample. This method of determining the denominator of the recovery calculation does not invalidate the results and has been used by other researchers in the field (2, 3, 4), including Borchardt (4). In fact, we were not able to find references, other than those associated with Borchardt et al. (5, 6), in which the concentration of the seed was determined in a seeded negative final concentrate created by filtering unseeded water with the same source and volume as the recovery test.

Although for some experiments the recovery trials were done over several days, we took great pains to collect new samples for each trial day and ensure that water quality did not change over the course of the trial (no rain event, high waves, etc.). We also do not believe that mixing seeded and naturally occurring microorganisms makes the filter comparisons uninterpretable, as Borchardt et al. suggest. All the filters in our trials were treated the same in that they had all had the same matrix effects and the same microorganisms (seeded and naturally occurring). Although there were more trials for enterococci and *Escherichia coli* for the glass wool and automatic ultrafiltration (UF) (because these filters were used for unseeded controls) and fewer trials for protozoan pathogens for the NanoCeram, we do not believe that the numbers of trials need to be exactly the same to validate our results.

We assume that the matrix effect and inhibition comments by Borchardt et al. are in regard to virus analyses since they refer to quantitative PCR (qPCR) determinations of the seed and qPCR inhibition values (which were only measured for viruses). Inhibition of qPCR was measured for each sample in our study. Instead of using hepatitis G virus (HGV) armored RNA as an inhibition control and assuming that inhibition of HGV is similar to that of other viruses, as was done by Lambertini et al. (5), we chose to seed a subsample of the final unseeded concentrate with the actual DNA and RNA viral targets. Multiple dilutions of these control samples were analyzed which permitted us to assess qPCR inhibition and choose an appropriate dilution of test sample to analyze.

We do not disagree that the variability rank score (RCV) is an *ad hoc* measure and that filtration methods with better recoveries are more resistant to shifts in the RCV-based ranks (R') relative to the recovery-based ranks (R) than are filtration methods with poorer recoveries. It is our opinion that recovery is of primary importance when assessing health risk from pathogens because failure to detect pathogens when present results in underestimation of exposure-related health risks. Ideally, microbial concentration methods will have both high recoveries and low variability. A concentration method that consistently results in low or no recovery of microorganisms can have low or zero variability, so variability alone is not a good measure of performance.

The RCV and the corresponding RCV-based ranks were computed in an attempt to develop a logical score that assesses recovery and variability. It results in scores that are strongly weighted in favor of recovery but with some penalty for high variability. Use of the RCV-based ranks did not change the rank order of any of the filtration systems ranked number 1 based on recovery alone and resulted in changes in the number 2-ranked systems for only 4 of the 9 microorganisms (see Table 2 in reference 1) (enterovirus, avian influenza virus, *Cryptosporidium*, and *Giardia*). In most cases where R' differed from R, the rank changed only by 1. The fact that methods with better recoveries are more resistant to changes in rank order than methods with poorer recoveries is consistent with the emphasis we place on recovery relative to variability.

Just as there are multiple ways to assess the "best" statistical model, there also are multiple ways to assess the "best" filtration method. While we make no claim that the RCV-based rank is definitive, we feel that its characteristics are both logical and appropriate and know of no other measure that offers significant advantages. Using this method, we were able to show that if one were targeting all types of microorganisms (bacteria, viruses, and protozoa) in lake-water samples and were limited to one filter type, automatic ultrafiltration might be the method of choice (see Table 3 in reference 1). If one were targeting one type of microorganism, our results could be used to select the most appropriate filter type for that microorganism.

Borchardt et al. further suggest that more established statistical methods should be used to evaluate filter performance. We did just that by comparing percent recoveries for each microorganism by filter type using the Tukey-Kramer multiple-comparison test

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(see Fig. 3 in reference 1). Discerning readers will be able to use these statistical results along with RCV-based ranks to select filtration methods for their own studies.

We agree that cost, ease-of-use, and quality-control requirements are very important factors for determining an appropriate filtration method. Cashdollar and Wymer (7) address these factors and point out that the glass-wool filters made by Borchardt's laboratory are not commercially produced but instead are hand packed. Although glass-wool filters are relatively inexpensive, the extent to which the small variations in packing that are likely to occur in a manual process might affect filter performance is uncertain. Taking these factors into account, plus the results of the filtration recovery comparisons, a system using a hollow-fiber filter such as the dialysis ultrafilters used in Francy et al. (8) or in our current study may be the most practical option for concentrating bacterial, protozoan, and viral pathogens simultaneously in lakewater samples. The latter filters are commercially available, provide a large filter surface area, and are made under quality control standards given their intended use in the medical field.

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This publication has been reviewed by the U.S. Environmental Protection Agency but does not necessarily reflect U.S. Environmental Protection Agency views. No official endorsement by the U.S. Environmental Protection Agency should be inferred. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. government.

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