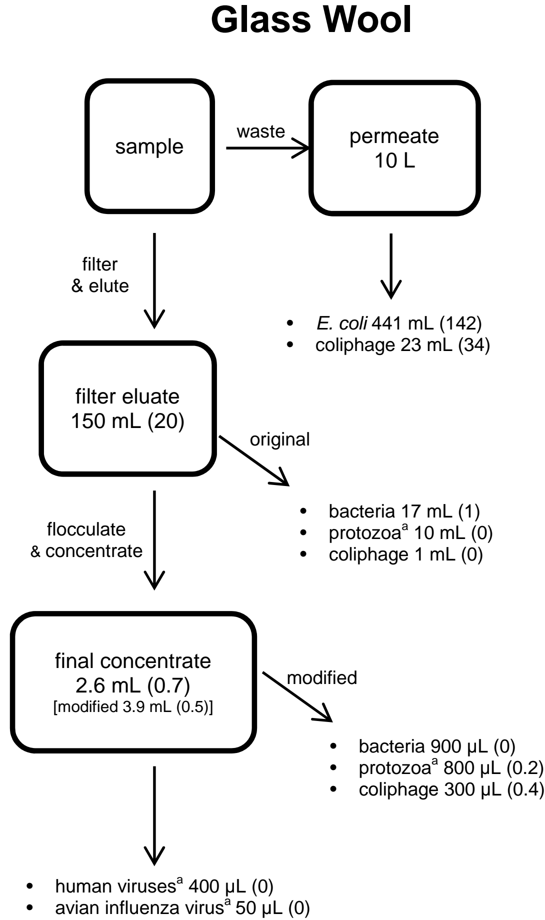
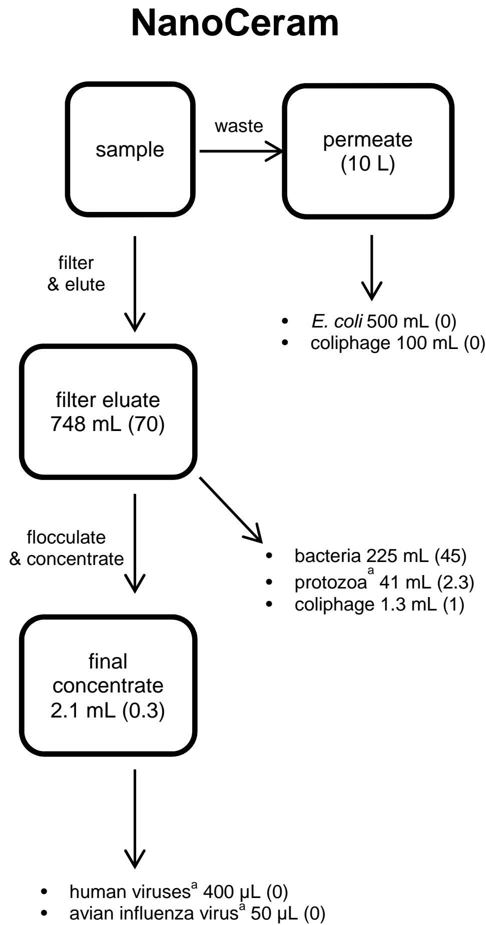
FIG S1 Glass-wool filtration method steps. The average volumes removed at each step (and SDs) are listed for each type of microorganism.



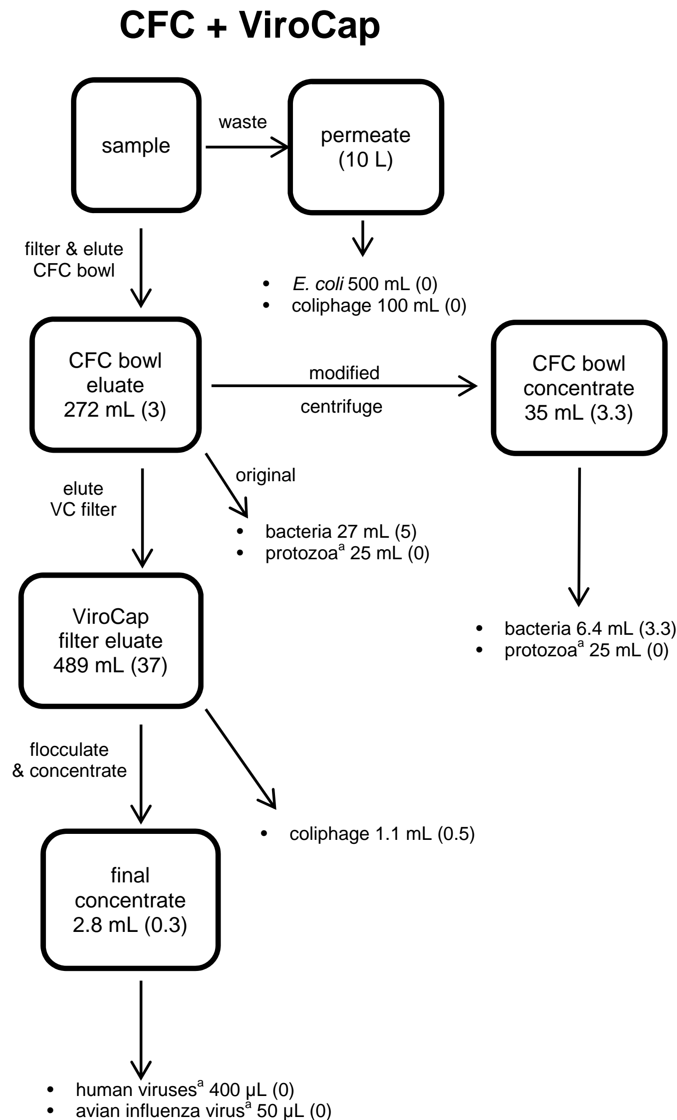
After the 10-L sample was pumped through the glass-wool fiber filter (1, 2), microorganisms were eluted from the filter with 140 mL of elution solution containing 3.0% beef extract made from BBL[TM] Beef Extract powder (BD, Franklin Lakes, NJ) and 0.5 M glycine at pH 9.5. The filter eluate (150 mL, average) was adjusted to pH 7.0-7.5 with 1 M HCl, and aliquots were removed for bacteria, coliphage, and protozoa analyses. The remaining filter eluate was flocculated with 16.0 g polyethylene glycol (PEG) and 2.3 g NaCl, stirred for 1 hour at 4°C, held overnight at 4°C, and then centrifuged at 3,300 × g for 1.5 hours. The pellet was resuspended in 2 mL of 0.15 M sodium phosphate. The resultant final concentrate was stored at -70°C for subsequent virus analysis. For samples collected at sites 5–7, a modified method was used (3) so that larger proportions of were analyzed for bacteria, coliphage, and protozoa. The modified method included only one change from the original method—2 mL (average) were removed for bacteria, coliphage, and protozoan analyses from the final concentrate (2.6 mL, average) instead of from the filter eluate in the original procedure. The modified method still included the same flocculation and concentration steps as the original method.

FIG S2 NanoCeram filtration method steps. The average volumes removed at each step (and SDs) are listed for each type of microorganism.



After NanoCeram filtration of the 10-L sample, microorganisms were eluted from the filter with 1 L of elution solution (1.5% beef extract, 0.05 M glycine, pH 9.0) (4). Then, 267 mL (average) of filter eluate was removed for bacteria, coliphage, and protozoan analyses and adjusted to pH 7.0–7.5. The remaining filter eluate was concentrated with diatomaceous earth (no longer manufactured, J.T. Baker Chemical Co, Phillipsburg, NJ) at pH 4.0, adjusted after gravity filtration to pH 7.0-7.5, flocculated with 4.0 g PEG and 0.57 g NaCl, then centrifuged to form a pellet. The pellet was resuspended in 2 mL of 0.15 M sodium phosphate. The resultant final concentrate (2.1 mL, average) was stored at -70°C for subsequent virus analysis.

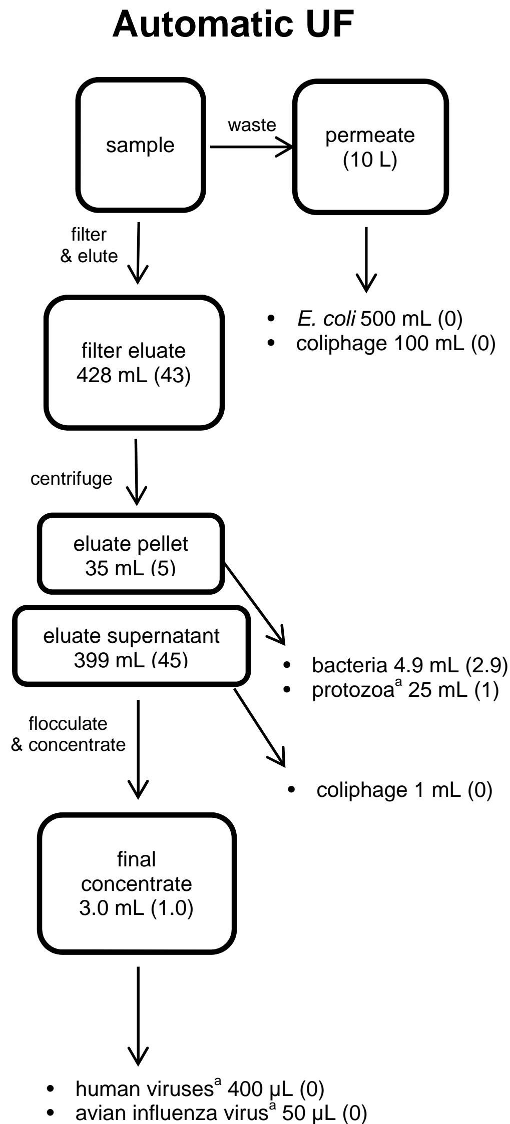
FIG S3 CFC+ViroCap filtration method steps. The average volumes removed at each step (and SDs) are listed for each type of microorganism.



The 10-L sample was pumped through the CFC bowl and ViroCap filter in sequence (5). Five mL of elution solution (1x PBS, 2.5 mL 1% Tween 80, and 22.5 mL sterile reagent water) was added to the CFC bowl, the bowl was placed on a wrist-action shaker for 15 min, and aliquots of the resultant CFC bowl eluate (272 mL, average) were removed for bacteria (27 mL, average) and protozoa (25 mL) analyses. Coliphage and viruses were eluted from the ViroCap filter with 500 mL OptimaRE® elution solution at pH 9 (Scientific Methods, Granger, IN). The filter eluate was adjusted to pH 7.0–7.5 and 1.1 mL (average) were removed for coliphage analysis. The remaining filter eluate was flocculated with 40 g PEG and 5.7 g NaCl and centrifuged to form a pellet. The pellet was resuspended in 2 mL of 0.15 M sodium phosphate. The resultant final concentrate (2.8 mL, average) was stored at -70°C for subsequent virus analysis. For samples

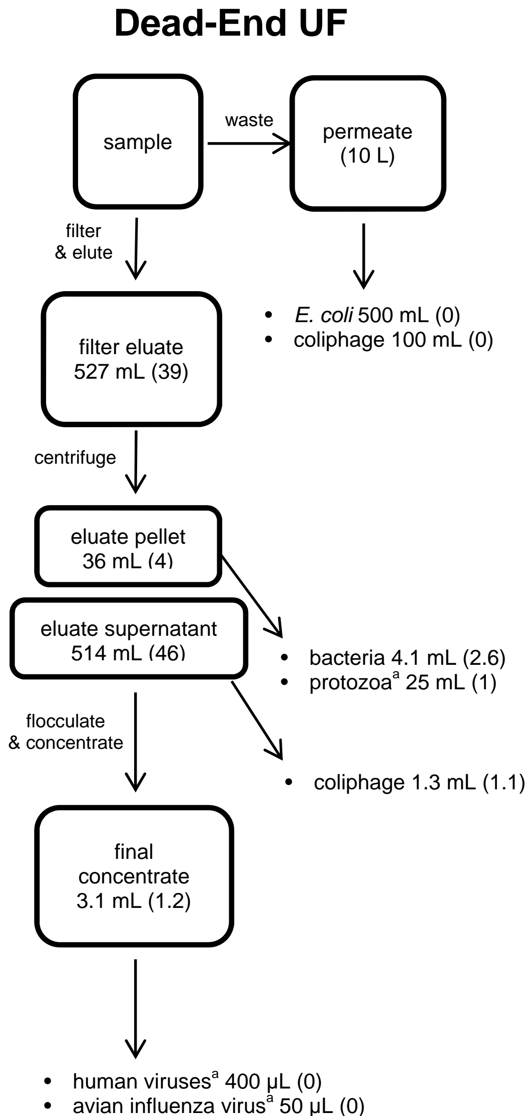
collected at sites 5, 6, and 7, a modified method was used so that larger proportions were analyzed for bacteria, coliphage, and protozoa. In the modified method, the CFC bowl eluate was concentrated by centrifugation at 3,300 x g for 30 min and aspirated down to 30 mL. The resultant CFC bowl concentrate (35 mL, average) was used for bacteria (6.4 mL, average) and protozoan (25 mL) analyses.

FIG S4 Automatic ultrafiltration method steps. The average volumes removed at each step (and SDs) are listed for each type of microorganism.



The automatic method operates on the principle of tangential-flow UF, with a computer controlled system automating the process of concentrating microorganisms. The 10-L sample was pumped through the automatic UF sampling device (6) per the manufacturer's instructions. After filtration, 1-L of elution solution (0.01% Tween 80) was recirculated through the sampling apparatus to remove microorganisms from the filter into an eluate bottle. The filter eluate was centrifuged at 3,300 x g for 30 min. The eluate pellet was resuspended to a volume (35 mL, average) that was sufficient for bacterial and protozoan analyses. One mL of the filter eluate supernatant was used for coliphage analysis. The remaining filter eluate supernatant was flocculated with 40 g PEG and 5.7 g NaCl and processed and stored to obtain a final concentrate for subsequent virus analysis.

FIG S5 Dead-end ultrafiltration method steps. The average volumes removed at each step (and SDs) are listed for each type of microorganism.



The 10-L sample was pumped through the dead-end UF (7). The dead-end UF differs from the automatic UF in that dead-end UF involves a single pass of the water that is not recirculated. For dead-end UF, a 500 mL backflush solution (0.5% Tween 80, 0.01% NaPP, and 0.001% antifoam Y-30 emulsion was used to elute microorganisms from the filter into an filter eluate bottle. The filter eluate was centrifuged at 3,300 x g for 30 min. The filter eluate pellet was resuspended to a volume (36 mL, average) sufficient for bacterial and protozoan analyses. A small portion of the eluate supernatant (1.3 mL, average) was used for coliphage analysis. The remaining eluate supernatant was flocculated with 40 g PEG and 5.7 g NaCl and processed and stored to obtain a final concentrate for subsequent virus analysis.

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