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A Comparison of Methods to Concentrate Viruses from Environmental Waters Using MS2 as a Model

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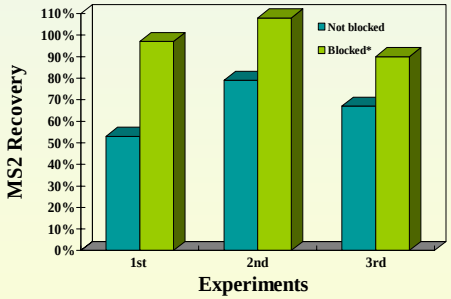
Abstract

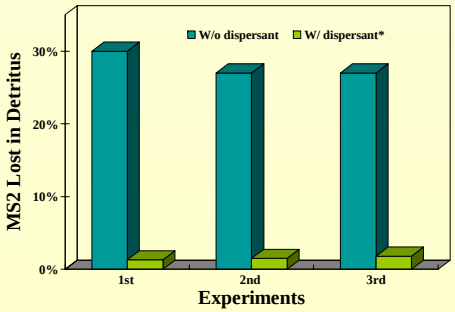
Viruses such as *Rotavirus*, *Adenovirus* and *Norovirus* are important etiological agents of gastroenteritis worldwide. With the high sensitivity and specificity of PCR, it is now possible to develop PCR-based methods to detect and quantify pathogenic viruses in environmental water samples. To develop reliable methods however, an effective procedure to concentrate viruses from large volumes of water is required. Because of the scale of concentration required, the procedure often requires two steps. The first to reduce tens of liters of water to less than half a liter and then a second to concentrate the sample to a final volume of less than 10 mL for RNA/DNA extraction. The objectives of the study were to compare the efficacy of hollow fiber ultrafiltration (HFUF) using F200B to that of an adsorption/elution method (AEM) using positively charged filters for concentrating viruses for the first step and to compare polyethylene glycol (PEG) precipitation to centrifugal ultrafiltration for the second step. A third objective was to determine the viral detection limit using real-time RT-PCR. Using beach water spiked with a single-stranded RNA bacteriophage (MS2) as a model, our results show a virus recovery rate of 84±6% and 18±8% for the HFUF method and AEM, respectively. For the second concentration step, we obtained a recovery rate of 49±5 % and 87±7% using PEG precipitation and centrifugal ultrafiltration, respectively. A potential limiting factor to more widespread using of HFUF is the higher cost and we found that cost can be reduced by using reusable filters. We were able to sanitize and reuse the same filter at least six times without affecting the virus recovery rate or the processing time.

Blocking hollow fiber filters with calf serum improves rate of virus recovery



* Blocked with 5% calf serum before use for 16 h at room temperature

Using a dispersant prevents adsorption of virus to detritus and enhances recovery



* With 0.01% sodium polyphosphate (NaPP)

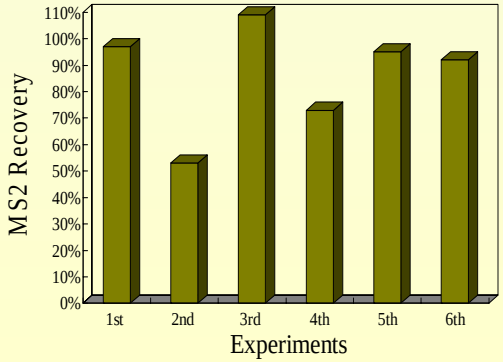
Water samples (10 L) spiked with MS2
↓
Continuous flow centrifugation
Outflow water — Detritus removed
Hollow Fiber Filtration — Virocap Filtration

MS2 recovery determined using plaque assays

Hollow fiber filters can be sanitized using 0.5% bleach

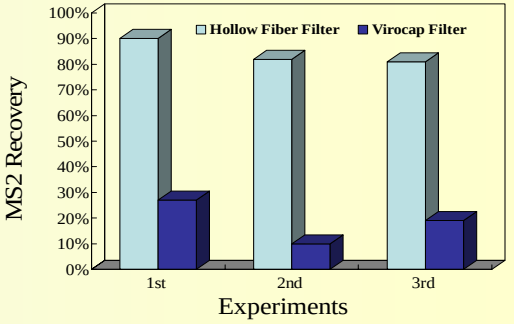
Experiment #	Input MS2 (PFU)	Sanitization time	Recovered MS2 (PFU)
1	1.9×10^7	10 min	0
		30 min	0
2	1.4×10^7	10 min	0
		30 min	0
3	2.6×10^7	10 min	0
		30 min	0

Sanitized hollow fiber filters can be reused at least six times

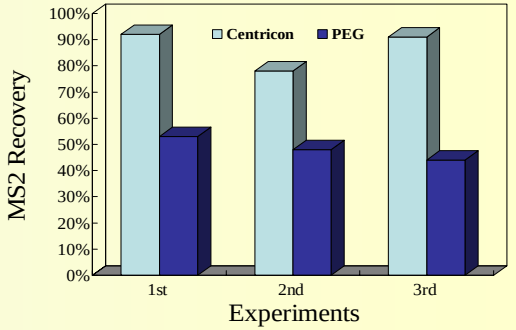


The hollow fiber filter was not blocked with calf serum in Experiments 2 and 4 resulting in lower MS2 recovery rates.

Rate of virus recovery was higher using hollow fiber filters compared to Virocap filters



Ultrafiltration is more effective than PEG precipitation for final virus concentration



Conclusions

- Blocking protein adsorption sites on hollow fiber filters with 5% calf serum before use results in ~ 30% increase in virus recovery.
- If detritus is first removed to prevent filter clogging, adding a dispersant such as sodium polyphosphate prevents virus adsorption to detritus and improves virus recovery efficiency.
- Substantially more virus is captured and recovered using filters that physically retain and concentrate the virus than by filters that capture virus by adsorption.
- Hollow fiber filters are expensive but can be sanitized and reused multiple times.
- To concentrate virus further, ultrafiltration is more effective than PEG precipitation.

Acknowledgements

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