

# Methods for Virus Recovery in Water

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## 1. INTRODUCTION

Research involving the recovery of viruses from various water sources can be traced back to the 1950s when scientists were eager to better understand the occurrence and transmission of disease-causing viruses in water; most significant viruses at the time were poliovirus and hepatitis virus. LoGrippto and Berger (1952) utilized ion-exchange resins to concentrate poliovirus, and shortly thereafter Kelly (1953) demonstrated the use of these same resins for the concentration of coxsackie viruses from sewage samples. This seminal research led to development of the first methods that relied on manipulation of virus surface charge for recovering viruses from large volumes of water. Virus adsorption to filter media followed by elution by a pH-adjusted solution (Wallis and Melnick 1967) became collectively known as VIRADEL (virus adsorption elution) method. Shortly after VIRADEL methods were introduced, alternative methods to concentrate viruses based on size exclusion (i.e. ultrafiltration based on nominal molecular weight cut-off) were investigated. Belfort et al. (1975) concentrated viruses from 5 L of water using hollow fiber membranes and from this initial report through the present day, numerous studies have shown hollow-fiber ultrafiltration is effective for the recovery of viruses from water (Fong and Lipp 2005; Gensberger and Kostić 2013; Hill et al. 2007).

At this point, two questions arise: (1) How are viruses in water related to viruses in food and (2) Why is it important? Simply stated, there is an intimate connection between food safety and water quality as water is used at almost every node in the food production process. Moreover, viruses are the primary cause of foodborne disease outbreaks (FBDO) in the United States and in most other high-income countries (EFSA BIOHAZ Panel 2011; Lopman et al. 2003; Scallan et al. 2011). From an epidemiological perspective, human noroviruses (HuNoVs) are the most significant in foodborne trans-

mission though other viruses of concern include hepatitis A virus (HAV), enteroviruses, and hepatitis E virus (HEV; Le Guyadar et al. 2008; Yugo and Meng 2013).

The most commonly reported connections between water quality and food safety are viruses in fresh produce and shellfish (i.e. bivalve mollusks such as oysters) (see Chaps. 6 and 7 in this book). For these foods, large volumes of water must be used during pre- and post-harvest processing. The ongoing conversation in shellfish production revolves around the relevance of using fecal indicator bacteria (*Escherichia coli*, *Enterococcus* spp.) as predictors of viral pathogen contamination when monitoring the quality of harvesting waters or shellfish meats (Richards et al. 2010). The issue arises because an indicator should correlate with the presence of pathogens; however, bacterial indicators have been repeatedly shown to correlate poorly with viruses (Field and Samadpour 2007). Fresh produce (i.e. fruits and vegetables) can become contaminated with waterborne bacteria and viruses during production via irrigation water, preparing and spraying pesticides, preventing dehydration, or during produce washing or cooling. The proposed agricultural water standards in the US FDA's proposed rule for produce safety rely on *E. coli* to indicate sanitary quality of water (USFDA 2014). Thus, the same limitations would apply regarding the often observed absence of a relationship between bacterial indicators and human pathogenic viruses.

Human enteric viruses may be introduced into the water environment through various routes including discharge of sewage-contaminated water into food production settings (i.e. oyster harvesting waters and surface water used for irrigation), land application of municipal biosolids, wastewater, or septage with subsequent runoff, and groundwater contaminated by infiltrating surface water, faulty septic systems or leaking sanitary sewers (Gibson 2014). Overall, data are limited on virus occurrence in water used for food production even though the industry's heavy reliance on water suggest it is a vehicle for dissemination of viruses in the food supply (Song et al. 2006; Stine et al. 2005).

There are several options for recovering viruses from water, although each method brings along its own set of challenges, primarily stemming from the low but significant levels of human enteric viruses that may be present in environmental water samples at sites located around the world (Dong et al. 2010; Gibson et al. 2012; Sinclair et al. 2009; World Health Organization 2009; Ye et al. 2012).

## 2. VIRUS RECOVERY METHODS

Wallis et al. (1979) originally prescribed the ideal method for virus recovery as being able to: (1) process large volumes (>100 L) of various types of water in the least amount of time; (2) consistently concentrate most types of viruses

present in water and wastewater, (3) be used easily and economically, and (4) recover viruses that are particle-associated or present in viral aggregates. In the subsections that follow, various methods are described for recovering and concentrating viruses from water.

## 2.1. VIRADEL

The first generation of VIRADEL methods was based on adsorption of viruses to electronegative (EN) membrane filters (Sobsey et al. 1973). However, because viruses have a net negative charge on their capsid surface at neutral pH, to facilitate virus adsorption to the negatively-charged filters, EN-based methods require cumbersome chemical modification of the water sample including acidification or addition of multivalent cations (Sobsey and Jones 1979). Shortly thereafter, Sobsey and Jones (1979) suggested that membrane filters with a more positive charge at the pH of natural waters and tap water (pH 5–9) would be more advantageous for virus recovery compared to EN-based methods. This initial investigation of electropositive (EP)-based membrane filter methods by Sobsey and Jones (1979) demonstrated a marked increase in virus recovery from tap water (e.g., <5 % recovery with EN filter vs. 64 % recovery with EP filter) and served as the basis for developing the standard method for virus recovery and concentration from large volumes of water (USEPA 2001).

Wallis et al. (1979) identified several factors influencing the adsorption of viruses to filter surfaces: filter surface charge and surface area resulting from the composition and design of the filter membrane; flow rate during sampling (i.e. high flow rate equates to less viruses adsorbed); ratio of pore diameter to virus diameter; pH; multivalent cations; and the presence of proteinaceous substances competing with viruses for adsorption. Filter surface charge plays a significant role in virus adsorption as demonstrated by the difference in viral recovery between EN- and EP-based methods. In addition, virus adsorption capacity decreases with decreasing filter surface area because there are a finite number of adsorption sites per unit area (Wallis et al. 1979). However, additions of acids (for pH manipulation) and salts (multivalent cations) can greatly increase filter adsorption capacity regardless of filter surface area; therefore, the virus adsorption capacity of filters is really never exceeded (Wallis et al. 1979). The impact of multivalent cations on filter charge is one of the primary reasons why EN-based methods outperform EP-based methods for the recovery of viruses from marine waters (Katayama et al. 2002; Lukasik et al. 2000). Overall, the net charge of the filter is one of the primary determining factors in virus adsorption.

Present day, researchers still use both EN- and EP-based methods for recovery of viruses from water though far more emphasis has been placed on EP-based methods. Table 10.1 summarizes the VIRADEL methods that are most commonly utilized for recovery of viruses, and Table 10.2 provides representative recovery efficiencies for each VIRADEL method by water type. For the EP-based methods, beef extract—glycine solutions at an alkaline pH is

most often used for virus elution from the filter membrane (Table 10.1). However, other eluents have previously been used including 5× nutrient broth (Hill et al. 1974), tryptose phosphate broth (Farrah et al. 1976), and amino acids—arginine and lysine (Farrah and Bitton 1978). The type of eluent is important to note as organic and inorganic compounds in beef extract solutions are known to inhibit reverse transcription (RT) for cDNA synthesis from viral RNA as well as PCR amplification (Abbaszadegan et al. 1993). This is important because many waterborne viruses are not readily culturable requiring downstream detection methods that rely on RT and PCR technologies.

After filtration with EN-based methods the filter is rinsed with a dilute acid followed by elution with alkaline buffer (pH > 9.0) containing either high salt concentration and surfactant (Hamza et al. 2009) or proteinaceous substances such as skimmed milk (Wyn-Jones et al. 2011). De Keuckelaere et al. (2013) compared four methods, two EN-based, one EP-based, and one using tangential flow filtration for recovery of five types of human enteric viruses from four irrigation water sources and processing water from a fresh cut lettuce processing plant. The investigators concluded the EN-based method including virus elution with high salt concentration buffer provided the highest virus recoveries across the range of water types tested. However, De Keuckelaere et al. (2013) reported much lower recovery efficiency for three of the four methods evaluated as compared to previously published reports.

A novel filter-less method was reported by Calgua et al. (2008) where 5–10 L volumes of seawater or freshwater amended with artificial sea salts are flocculated with 1 % (w/v) skimmed milk solution at pH 3.5. Samples are stirred for 8 h, the floc is allowed to settle for another 8 h, and then centrifuged. Mean virus recoveries from freshwater were in the range of 40–50 % (Calgua et al. 2013). The EN-based methods and skimmed milk method may be considered advantageous when compared to EP-based methods and ultrafiltration based on the volume of water needed. For instance, only 2–10 L (Fong et al. 2005; Lee and Kim 2008; Hamza et al. 2011) of a given water sample can be concentrated to detect viruses in the former methods; however, given the same water sample, hundreds of liters may need to be concentrated by EP-based and ultrafiltration to detect the viruses present. Simultaneous concentration of PCR inhibitors and particulates during large volume filtration is speculated to offset the benefits of concentrating more viruses (Albinana-Gimenez et al. 2009; De Keuckelaere et al. 2013), but why small sample volumes appear to provide the same limits of virus detection has not been systematically investigated. Confounder effects, such as correlation between the method selected for sampling and virus concentrations in the waters being sampled, or between sampling method and the use of nested PCR for virus detection, are possible considerations.

The EP-based filtration method described by Sobsey and Jones (1979) served as the basis for USEPA to prescribe using the positively-charged 1MDS filter for virus concentration from water for the Information Collection Rule (USEPA 2001). Although effective (Table 10.2), the 1MDS method has

**Table 10.1** VIRADEL (Virus Adsorption—Elution) methods for recovery of viruses from water samples

		VIRADEL filtration methods				
	IMDS <sup>a</sup>	NanoCeram <sup>®b</sup>	N66 Posidyne	Glass wool	HA—membrane	
Principle	EP	EP	EP	EP	EN	
Water type <sup>c</sup>	S, G, D, E, W	S, G, D, E	S, D	S, G, D, E, Ag, W	S, G, D, E	
Volume (L) <sup>d</sup>	1—>1000	10—>1900	20—>200	2—>100,000	0.2–20	
Sample/filter amendments	Adjust sample pH if >8.0	None	None	Adjust sample pH if >7.5	Sample pH 3.5–4.0 Or Precondition filter	
Pre-filter	Optional	Optional	Optional	Optional	Optional	
Elution	Beef extract—glycine (pH 9.5)	Beef extract—glycine (pH 9.0–9.5); sodium polyphosphate—glycine (pH 9.3)	Beef extract (pH 9.5)	Beef extract—glycine (pH 9.5)	Ti-alk (pH 9.2); skimmed milk glycine solution (pH 9.5); TGBE (pH 9.5)	
References	Cashdollar and Dahling (2006), Gilgen et al. (1997), Lee et al. (2011), Polaczyk et al. (2008), Rose et al. (1984), and Sobsey and Glass (1980)	Cashdollar and Wyrmer (2013), Gibbons et al. (2010), Ikner et al. (2011), Karim et al. (2009), and Lee et al. (2011)	Cashdollar and Dahling (2006), Shields et al. (1986)	Environment Agency. (2000), Francy et al. (2013), Gibson et al. (2012), Grabow and Taylor (1993), Lambertini et al., (2008, 2011), Millen et al., (2012), and Vilagines et al. (1997)	De Keuckelaere et al. (2013), Farrah et al. (1976), Haramoto et al. (2009, 2012); and Victoria et al. (2009)	

EP electropositive, EN electronegative, HA mixed cellulose esters, ND not done; Tr alk 0.05 M KH<sub>2</sub>PO<sub>4</sub>, 1.0 M NaCl, 0.1 % (v/v) Triton X-100, TGBE 100 mM Tris, 50 mM glycine, beef extract 1 % (w/v), S surface water, G groundwater, D drinking water, E salt water, Ag agri-culture runoff, W wastewater

<sup>a</sup>Zetapor<sup>®</sup> or Virosoorb<sup>®</sup>

<sup>b</sup>Both cartridge and disc filter options are available, but only NanoCeram<sup>®</sup> Virus Sampler cartridge filters are referred to here. In addition, ViroCap is the name of the self-contained cartridge format of NanoCeram<sup>®</sup>

<sup>c</sup>As reported in published literature

<sup>d</sup>Range of water volumes for all water types; values based on published research

**Table 10.2** Virus recovery efficiencies for VIRADEL filtration methods by water type<sup>a,b</sup>

	% Recovery <sup>c</sup>				
	Surface water	Groundwater	Tap water	Seawater	Wastewater
1MDS	36–100	62–72	33–67	40–53	14–61
NanoCeram <sup>®</sup>	38–65	86	14–84	<3–>96	ND
Glass wool	5–72	8–56	28–98	15–99	52–62
HA-membrane	15–67	ND	3–80	3–53	ND

ND not done

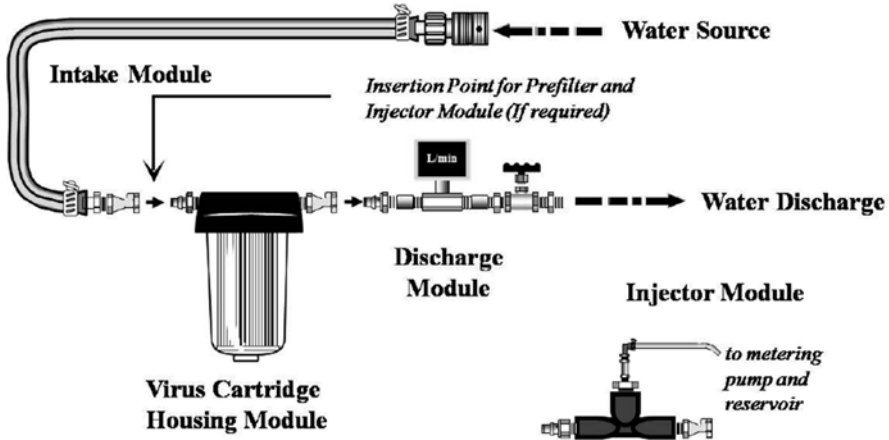
<sup>a</sup>References listed in Table 9.1 apply here as well

<sup>b</sup>Viruses tested include; adenoviruses (type 2, 40,41), human astrovirus GI, coxsackie virus B5, echoviruses (type 1, 7, 18), Hepatitis A virus, MS2 bacteriophage, murine norovirus, human norovirus (GI and GII), phi X174 bacteriophage, polioviruses (LSc, Mahoney, Sabin strains), human rotavirus

<sup>c</sup>Recovery percentages listed are based on primary concentration followed by detection of viruses by either culture-based or molecular-based methods

been criticized for being cost prohibitive for routine monitoring purposes—each filter cartridge costs approximately \$200–300 (Cashdollar and Wymer 2013). In 2010, USEPA published Method 1615, a revised method for detecting enteroviruses and noroviruses in water that permits virus concentration by the positively charged NanoCeram<sup>®</sup> filter, which cost roughly 20 % (\$40–60) of the cost of the 1MDS filter and is equally as effective for these two viruses (Fout et al. 2010). The Nanoceram filter does not appear to be as effective for concentrating adenoviruses (Gibbons et al. 2010; Ikner et al. 2011; Pang et al. 2012; McMinn 2013).

Glass wool filtration is another effective EP-based method (Table 10.1). Oiled sodocalcic glass wool is washed in series with 1 N HCl, distilled water, 1 N NaOH, and a final wash with distilled water until a neutral pH is achieved. The washed glass wool can be stored in sterile phosphate buffered saline for up to 1 week at 4 °C. Filters are made by packing washed glass wool tightly into column housings with size and fittings appropriate for the virus sampling plan at hand (Vilagines et al. 1993; Millen et al. 2012). Filter construction configurations are highly flexible for specific projects and can be used to filter large volumes of water (Gibson et al. 2012; Lambertini et al. 2008; Vilagines et al. 1997). Similar to other EP-based filter cartridges, glass wool requires the use of an alkaline eluent such as a beef extract—glycine solution. Glass wool filters are a fraction of the cost—\$4.40 per filter (not including labor)—of NanoCeram<sup>®</sup> and 1MDS filters (Lambertini et al. 2008) and are demonstrated to achieve similar virus recovery efficiencies (Table 10.2). The primary disadvantage of glass wool filters is that they are not commercially available, lending to the perception that hand-packing in the laboratory may lead to filter-to-filter variability in virus recovery efficiency (Cashdollar and Wymer 2013). Even then, the United Kingdom Environment Agency utilizes the glass wool filtration technique in standard methods for the recovery and concentration of viruses in groundwater (Environment Agency 2000).

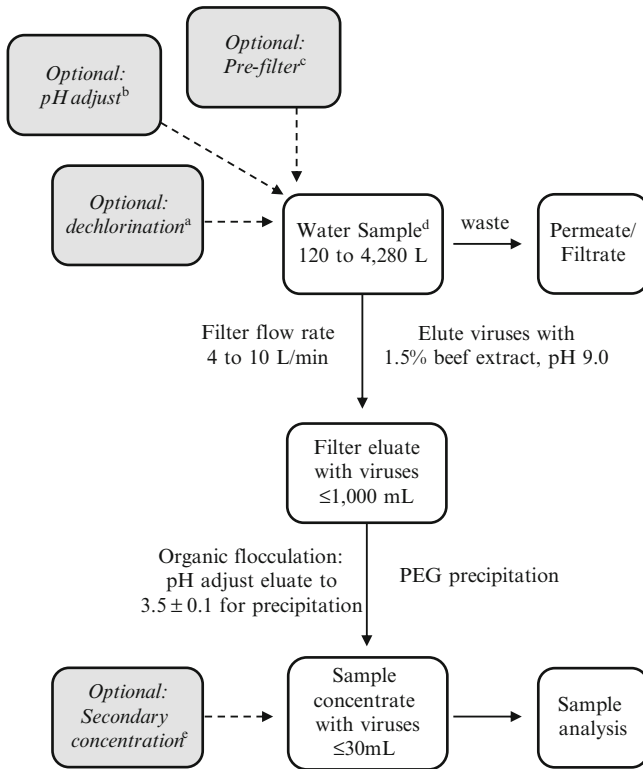


**Fig. 10.1** Basic setup for recovery of viruses from water using electropositive (EP)-based methods. An injector module is used for dechlorination if disinfectant present in the water source and/or for pH adjustment. A 10- $\mu\text{m}$  polypropylene prefilter may be required if the water sample turbidity is elevated.

As described in USEPA Method 1615, the basic setup and steps for recovering viruses from tap water using an EP-based cartridge filtration method are shown in Figs. 10.1 and 10.2, respectively. Overall, when using EP filters such as NanoCeram<sup>®</sup>, 1MDS, or glass wool, the steps taken to recover viruses from a given water sample will depend on the need to neutralize chlorine residual disinfectant, adjust pH to the optimal level for virus adsorption, and add a pre-filter for highly turbid samples.

## 2.2. Hollow Fiber Ultrafiltration

Hollow fiber ultrafiltration (HFUF) for virus concentration and recovery from water was initially investigated by Belfort et al. (1975). There was a renewed interest in this method a decade ago for its potential to concentrate not only viruses but also other classes of microorganisms. Ultrafiltration methods rely on size exclusion for virus concentration rather than adsorption and hence difficulties associated with adsorbing viruses to various filter media are avoided. Ultrafilters are rated by molecular weight cut-off (MWCO) ranging from 1 to 300 kilodaltons (kDa), corresponding to a nominal pore size between 5 and 35 nm (Pall Corporation 2015). There are many different shapes of ultrafilter membranes available including spiral-wound, flat sheets, flat discs, and hollow fibers; the latter is the most commonly used shape for waterborne virus concentration. The shape of the filter impacts the membrane surface area available for filtration. Most hollow fiber membrane filters used for recovery of viruses range from 1.3 to 2.5 m<sup>2</sup> (Table 10.2). Ultrafiltration membranes are constructed from a variety of polymers such as polysulfone, polyacrylonitrile, and cellulose triacetate and can be used in two different modes; tangential (cross) flow (TF) and direct (dead-end) flow (DE).

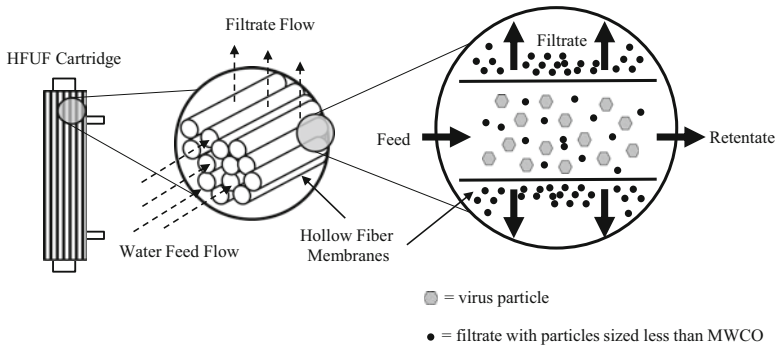


[Adapted from Fout *et al.* (2010) and Francy *et al.* (2013)]

**Figure 10.2** Virus Adsorption—Elution (VIRADEL) filtration method procedure. <sup>a</sup>If the water sample has a disinfectant present based on measured chlorine residual, then it must be dechlorinated with 2 % sodium thiosulfate using the optional injector module (Fig. 10.1) prior to filtration. <sup>b</sup>If the water sample has a pH >9.0 or >8.0 for NanoCeram<sup>®</sup> and 1MDS Zetapor<sup>®</sup> filter cartridges, respectively, then the pH must be adjusted with 0.12 M HCl until it measures 6.5–7.5. <sup>c</sup>If the water sample turbidity is >20 NTU or >50 NTU for NanoCeram<sup>®</sup> and 1MDS Zetapor<sup>®</sup> filter cartridges, respectively, then a 10- $\mu$ m polypropylene prefilter cartridge should be added prior to filtration but after dechlorination and pH adjustment. <sup>d</sup>The volume of water filtered depends on the water source and flow rate (Fout *et al.* 2010) <sup>e</sup>Various secondary concentration steps may be applied as described in Sect. 2.3 of this Chapter.

In TF filtration, a portion of the water (i.e. “feed” water) recirculates back to a central reservoir while the remaining portion passes through the membrane. The feed water is continuously recirculated until only a concentrate remains. Conversely, direct flow filtration pushes water through the membrane without recirculation, thus increasing the likelihood of membrane foul-





**Figure 10.3** Diagram of tangential flow ultrafiltration using a hollow fiber membrane cartridge filter. *HFUF* hollow fiber ultrafiltration.

ing, or the loss of membrane permeability that occurs due to accumulation of aquatic substances on or inside the membrane (Huang et al. 2008). During TF-HFUF, an applied pressure serves to force a portion of the feed water through the membrane to the filtrate side while viruses are concentrated in the retentate as opposed to being filtered out (Fig. 10.3). In TF, the retained components do not build up at the surface of the membrane but instead are swept along by the cross directional flow, dramatically reducing fouling and decreasing the tendency for microbes to adhere to the filter surfaces (Huang et al. 2008; Morales-Morales et al. 2003).

In DE filtration, water is not recirculated but flows directly through the filter, retaining viruses and other particulates greater in size than the MWCO. Ultrafilters are attached directly to a pressurized water source (e.g. drinking water distribution system), or in unpressurized systems (e.g. irrigation canal), a peristaltic pump is used. Input pressure less than 20 lb/in<sup>2</sup> is recommended (Mull and Hill 2012; Smith and Hill 2009). Dead-end HFUF may be advantageous in field settings where set-up of TF-HFUF equipment can be cumbersome and filter clogging is not problematic as in low turbidity water.

Belfort et al. (1975, 1983, 1985) initially investigated ultrafiltration for recovery of viruses in water in the mid-1970s to 1980s and interest was renewed in the 1990s with the potential ultrafiltration afforded for recovering multiple classes of microorganisms (pathogenic viruses, bacteria, and protozoa) with a single sampling method. Kfir et al. (1995) first demonstrated the simultaneous recovery of viruses and protozoa from 10 L water samples using 50 kDa MWCO ultrafiltration disc membranes. Moreover, the study showed that UF provided higher, more consistent recovery of enteric viruses (e.g., 63–100% with an average of 82%) when compared to traditional VIRADEL methods.

Additional studies by Morales-Morales et al. (2003) and Hill et al. (2005) switched the focus to hollow fiber ultrafilters operated in tangential flow for

the recovery and concentration of viruses in water. In Morales-Morales et al. (2003), two different hollow fiber ultrafilters with 50 kDa MWCOs were evaluated to recover microorganisms in 2 and 10 L water samples (tap water, groundwater, and surface water). Reported recoveries for PP7 and T1 bacteriophage were 31–74 % for 10 L surface water samples (Morales-Morales et al. 2003). Similarly, Hill et al. (2005) described a TF-HFUF method for the simultaneous recovery of microbes in tap water using commercially available dialysis filters with hollow fiber membranes (Table 10.3). The process was optimized through addition of (1) sample amendments such as sodium polyphosphate (NaPP); (2) an alternative membrane blocking procedure (e.g., NaPP solution as opposed to calf serum as described in Morales-Morales et al. (2003)); and (3) a backwashing step with a solution containing Tween 80 (Hill et al. 2005). Polyphosphates increase the negative surface charge of microbes suspended in water, creating surface charge repulsion. Tween 80 serves as a surfactant for reducing hydrophobic interactions between the filter surface and microbes, reducing adsorption of microbes to the hollow fiber membrane surface. Average recovery efficiencies for viruses using the optimized TF-HFUF method were 91 and 49 % for MS2 bacteriophage and echovirus 1, respectively (Hill et al. 2005).

Shortly thereafter, Hill et al. (2007) published another study on the concentration of viruses from 100 L samples of tap water. This study is the foundation for the development and optimization of additional TF-HFUF methods for concentration of viruses from 100 L water samples (Polaczyk et al. 2008; Hill et al. 2009; Francy et al. 2009; Gibson and Schwab 2011; Rhodes et al. 2011; Liu et al. 2012; Wu et al. 2013). Based on these studies, the basic steps for recovering viruses and other classes of microbes from water using HFUF are shown in Fig. 10.4, and the configuration for TF-HFUF is shown in Fig. 10.5a.

DE-HFUF methods gained attention due to the relative ease of field deployment compared to the TF-HFUF setup requirements. Olzewski et al. (2005) initially compared DE-HFUF to tangential flow ultrafilter cassettes (i.e. not hollow fiber dialyzers) and demonstrated similar recovery efficiencies (>65 %). However, high turbidity samples caused membrane fouling (or clogging) of the DE-HFUF (Olzewski et al. 2005). Smith and Hill (2009) demonstrated the recovery of diverse microbes including viruses from 100 L tap water and from mock surface water samples (i.e. tap water spiked with surface water to obtain a turbidity of 5 NTU) using DE-HFUF. The reported recovery efficiencies using DE-HFUF were similar to TF-HFUF (Table 10.4), making DE-HFUF an acceptable option for field-based sampling efforts. Additionally, Smith and Hill (2009) determined that Asahi Kasei REXEED 25S ultrafilters were better suited for use in DE-HFUF set-ups based on their hydraulic performance (i.e. ability to maintain an acceptable permeate flow rate at consistent, low system pressures) as compared to Exeltra Plus 210, F200NR, and REXEED 21S filters (Table 10.3).

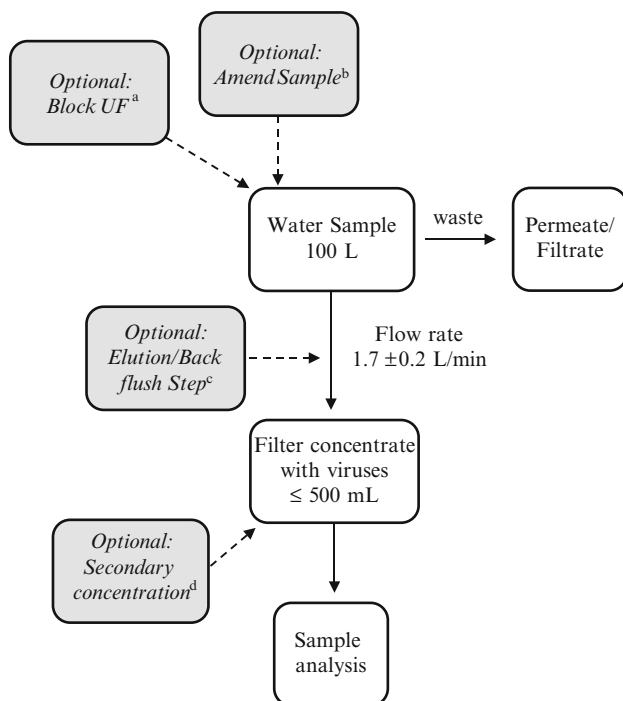
Mull and Hill (2012) also investigated DE-HFUF for recovery of viruses from medium (approx. 50 NTU) to high (approx. 100 NTU) turbidity surface

**Table 10.3** Hollow fiber ultrafiltration membranes used for virus recovery<sup>a</sup>

<i>Vendor</i>	<i>Model</i>	<i>MWCO (kDa)</i>	<i>Membrane composite</i>	<i>SA (m<sup>2</sup>)</i>	<i>Fiber ID (μm)</i>	<i>References</i>
Asahi Kasei	Rexbrane REXEED™_25S	30	Polysulfone	2.5	185	Francy et al. (2009, 2013), Knappett et al. (2011), Mull and Hill (2012), Rhodes et al. (2011), Smith and Hill (2009), and USEPA and CDC (2011)
Baxter	Exeltra Plus 210	70	Cellulose triacetate	2.1	200	Gibson and Schwab (2011), Liu et al. (2012), and Smith and Hill (2009)
Fresenius Medical	F200NR	30	Polysulfone	2.0	200	Hill et al. (2007, 2009, 2010), Liu et al. (2012), Polaczyk et al. (2008), and Smith and Hill (2009)
Medivators Inc.	F80A	30	Polysulfone	1.8	200	Hill et al. (2005) Polaczyk et al. (2008), Wu et al. (2013)
Pall Corp.	Hemacor HPH® 1400	65	Polysulfone	1.3	200	Francy et al. (2009), Liu et al. (2012)
	Microza (AHP-0013)	50	Polyacrylonitrile	0.017	800	Morales-Morales et al. (2003), Rajal et al. (2007), and Winona et al. (2001)

*MWCO* molecular weight cut-off, *SA* surface area, *ID* inner diameter

<sup>a</sup>Table adapted from Holowecy et al. (2009)



[Adapted from Francyet al. (2013)]

**Fig. 10.4** Hollow fiber ultrafiltration (HFUF) method steps. *UF* ultrafilter. <sup>a</sup>The ultrafilter may be blocked to reduce the potential for virus adsorption to the membranes; however, the need for blocking may depend on the brand of ultrafilter and water type (Liu et al. 2012). Blocking procedures may include (1) overnight incubation with 5 % calf serum (Hill et al. 2005); (2) recirculation of 500 ml 5 % calf serum for 5 min through the UF; (3) recirculation of 500 ml of blocking solution containing 0.055 % Tween 80, 0.001 % Antifoam A, and 0.1 % NaPP through the ultrafilter for 5 min (USEPA and CDC 2011); or (4) filtration of 1 L 0.1 % NaPP through the UF at 1.7 L/min with no back pressure (Hill et al. 2005). <sup>b</sup>Sample may be amended with sodium polyphosphate (NaPP) to a final concentration of 0.01 % prior to beginning filtration. <sup>c</sup>Following filtration, the ultrafilter can be eluted, or back flushed in the case of DE-HFUF, to capture any viruses that may be bound to the membrane. Elution buffers are recirculated within the TF-HFUF system and may include a solution containing 0.001 % Tween 80 or a solution containing 0.01 % Tween 80, 0.01 % NaPP, or 0.0001 % Y-30 antifoam emulsion. On the other hand, backflushing buffers are pumped through the permeate/ filtrate port and may include solutions containing slightly higher concentrations of components found in the elution buffers used in TF-HFUF, such as 0.5 % Tween 80, 0.01 % NaPP, and 0.001 % Y-30 antifoam emulsion. <sup>d</sup>Various secondary concentration steps may be applied to the filter concentrate as described in Sect. 2.3 of this Chapter.

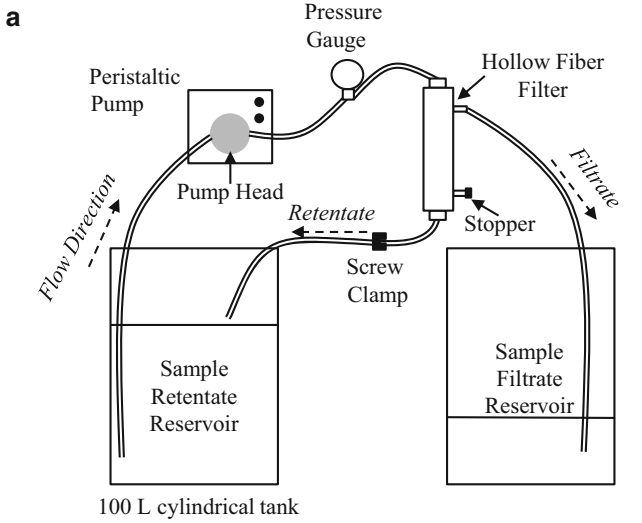
waters. There was a decrease in virus recovery with increasing turbidity levels; however, the recovery rates were still deemed acceptable (Table 10.4). Aside from differences in filtration configurations, DE-HFUF differs from TF-HFUF in the back flushing step. For DE-HFUF, an elution buffer is passed back through the permeate/filtrate port to recover viruses as opposed to recirculation of an elution buffer as done in TF-HFUF. The configuration for DE-HFUF based on Smith and Hill (2009) is shown in Fig. 10.5b and the basic steps are outlined in Fig. 10.4.

### 2.3. Secondary Concentration

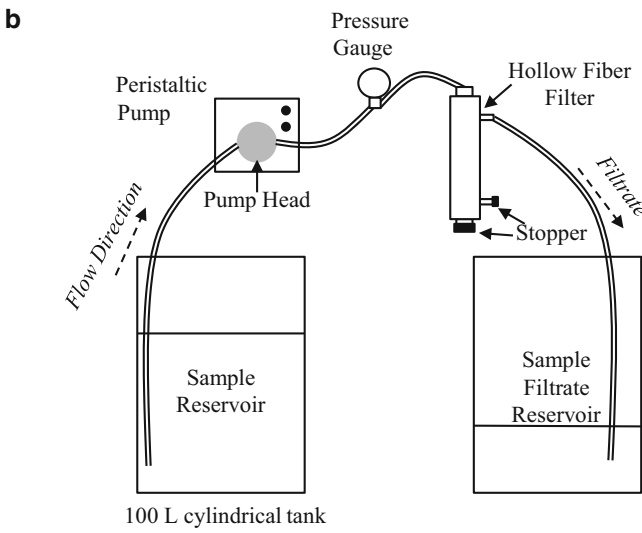
Even though HFUF and VIRADEL methods can concentrate viruses from water by 200- and >4000-fold, respectively, downstream methods for detection of viruses often require a secondary concentration and purification step after the initial concentration step. This additional step is needed since organic compounds (mostly humic acids) and metallic ions are often adsorbed or concentrated along with the viruses during primary concentration. These compounds can have inhibitory effects on downstream detection methods such as RT-PCR and real time qPCR. The methods most commonly used for secondary concentration and purification can be roughly categorized into one of the following groups: flocculation (organic or chemical), polymer-based hydroextraction, and centrifugal ultrafiltration.

One common method for secondary concentration is organic flocculation of the filter eluate (eluent + viruses) obtained during VIRADEL methods. This concept was originally proposed by Katzenelson et al. (1976) and involves the acidification of the eluate to pH 3.5 to cause flocculation of proteins, thus trapping any viruses present. The suspended floc is then centrifuged to a pellet followed by resuspension in 30 ml of sodium phosphate buffer. Organic flocculation is the method currently prescribed by USEPA Method 1615 for the concentration and processing of waterborne viruses (Fout et al. 2010). Additional flocculation methods include lanthanum-based chemical flocculation (Zhang et al. 2013) and celite (diatomaceous earth) concentration (Dahling and Wright 1986; Fout et al. 2003; McMinn et al. 2012, 2013).

Other effective methods for secondary concentration of viruses from water include polyethylene glycol (PEG) hydroextraction (Ramia and Sattar 1979; Lewis and Metcalf 1988; Schwab et al. 1996; Huang et al. 2000; Lambertini et al. 2008; Lee et al. 2011) and centrifugal ultrafiltration devices (e.g., Centricon Plus-70) (Hill et al. 2007; Gibson and Schwab 2011; Ikner et al. 2011). Hydroextraction with PEG has been paired with both VIRADEL and HFUF primary concentration methods, whereas centrifugal filtration devices have primarily been used in conjunction with HFUF. As with the primary methods for recovery of viruses, all secondary concentration steps have both advantages and disadvantages including cost, reproducibility, and ease of use, etc.



[Adapted from Hill *et al.* (2007) and USEPA & CDC, 2011]



[Adapted from Smith & Hill, 2009]

**Fig. 10.5** Basic setup for recovery of viruses from water using hollow fiber ultrafiltration (HFUF). (a) Tangential flow HFUF (TF-HFUF) (b) Dead-end HFUF (DE-HFUF).

**Table 10.4** Virus recovery efficiencies<sup>a</sup> for hollow fiber ultrafiltration methods by water type

<i>Reference</i>	<i>Flow setup</i>	<i>Water type</i>	<i>Volume (L)</i>	<i>Virus type</i>	<i>% Recovery</i>
Olszewski et al. (2005)	DE	G	100	PP7	70
				T1	71
				Poliovirus type 2	82
		S		PP7	86
				T1	70
				Poliovirus type 2	69
Hill et al. (2007)	TF	D	100	MS2	97
				φX174	71
Smith and Hill (2009)	DE	S <sup>b</sup>	100	MS2	73
Rhodes et al. (2011)	TF <sup>c</sup>	D	100	Poliovirus type 2	104
				MS2	99
Gibson and Schwab (2011)	TF	D	100	Murine NoV type 1	74
				MS2	48
				PRD1	57
		S		Murine NoV type 1	41
				Poliovirus type 2	28
				MS2	66
				PRD1	62
Liu et al. (2012)	TF	Reclaim	100	MS2	95 <sup>d</sup>
				φX174	90 <sup>d</sup>
Mull and Hill (2012)	DE	S <sup>e</sup>	100	MS2	66

*DE* dead-end, *TF* tangential flow; *S* surface water, *G* groundwater, *D* drinking water, *Reclaim* treated, reclaimed wastewater; *NoV* norovirus

<sup>a</sup>Recovery percentages listed are based on primary concentration followed by detection of viruses by either culture-based or molecular-based methods

<sup>b</sup>Tap water amended with SW to achieve 5 NTU

<sup>c</sup>Results from low rate filtration (<1900 ml/min) are reported here

<sup>d</sup>Combined high and low seed recovery efficiency

<sup>e</sup>Average over low (16 NTU), medium (46 NTU), and high (92 NTU) turbidity levels; however, recovery efficiency did decrease with increasing turbidity

### 3. METHOD SELECTION: WHAT IS IMPORTANT?

Despite differences in the details of elution and secondary concentration steps for various virus filters, the costs for reagents and disposables are about the same; approximately \$5.00–10.00 per sample with the exception of secondary concentration by centrifugal UF devices which adds \$30 or more per sample. Processing times, however, can differ greatly among the elution and

secondary concentration procedures and depend also on the laboratory equipment and personnel available for processing filters in batches. Time necessary for clean-up and equipment sterilization must also be considered.

What, then, are the criteria for selecting a filtration method for virus sampling? Several criteria are important. (1) If in addition to viruses, concentrating bacteria and protozoa is required, ultrafiltration and glass wool filtration are good choices because there is sufficient evidence to indicate that these methods effectively concentrate a variety of microbial targets. (2) Sample flow rate can be important when there are many sample sites, long travel times between sites, and limited field sampling personnel. The Nanoceram<sup>®</sup> filter at flow rate of 10 L/min is advantageous in that respect, requiring one third less time than DE-HFUF at 2.9 L/min. (3) For water samples with pH exceeding 7.5, if the standard approach of decreasing pH inline during sampling is not workable, glass wool filtration should be avoided. However, Nanoceram<sup>®</sup> filters are reported to be effective at pH values of up to pH 9.0. (4) Tangential flow ultrafiltration is not always practical in field settings, although the requirement for large sample volumes to be transported to the laboratory, might also be impractical. (5) DE-HFUF is prone to clogging and virus recovery has been shown to decrease with increases in turbidity. (6) Lastly, intangible factors such as experience and confidence with equipment and technical procedures should be considered when making a decision on selection of virus filter.

Regardless of filtration method, recovery controls are essential to demonstrate the chosen method does, in fact, concentrate the target microbes from the water being sampled. The general approach is to transport a test water volume (e.g., 10–20 L) to the laboratory, seed it with quantified target, filter and process the water, quantify the target in the final concentrate, and calculate the percent of target recovered. Another approach, more representative of field settings and typical for large sample volumes (hundreds of liters), is to filter the sample at the field site with a target volume minus 10 L, transport the remaining 10 L back to the laboratory, seed it with the target microbes, and finish filtration with the same filter from the field (Fout et al. 2010). A companion volume of unseeded test water should also be filtered in order to quantify indigenous target microbes present in the water. This quantity is then subtracted from the numerator of the recovery calculation.

In addition, when recovery is measured by qPCR, a third companion volume of unseeded test water should be filtered and processed identically as the seeded test water. The target microbial seed is then added to the final concentrate created from this third volume and quantified by qPCR. This quantified value is used as the denominator for calculating virus recovery. This step is crucial for accuracy because filtration and the downstream processing steps can affect qPCR inhibition or other water constituents that shift quantification cycle ( $C_q$ ) values (Borchardt et al. 2013). Lambertini et al. (2008) observed that inhibition during qPCR was detected in the beef extract eluent from the sample spiked with microbes processed by glass wool filtration; however, qPCR inhibition was not detected in the mock beef extract eluent spiked with



microbes indicating that constituents in the water concentrated during filtration contained inhibitors. In this instance, qPCR inhibition underestimated the quantity of microorganisms spiked in the water sample, resulting in an overestimate of virus recovery. Using six viruses, Calgua et al. (2013) demonstrated that percent recoveries varied depending on which step, and consequently which matrix, in the concentration process the viral seeds were quantified.

Ideally, a recovery control would be performed for each sample, but that is impractical in terms of cost and time. Water matrix type (i.e., the combined attributes of turbidity, pH, dissolved organic matter, inorganic compounds, divalent cations, etc.) is an important determinant of recovery for both VIRADEL and HFUF methods (Hill et al. 2007; Lambertini et al. 2008; Mull and Hill 2012; Wu et al. 2013). Insofar as the waters to be sampled are known to vary in their matrices, for example by location, season, or aquifer type, the number of recovery controls can be planned to correspond with these various matrices. However, this number of recovery controls can be impractical as well and ultimately the number of controls is decided in the context of the sampling plan goals and whether potentially false-negative samples can be tolerated. At a minimum, several recovery controls should be performed during the sampling period just to ensure the procedures in the laboratory are working, particularly if there is a change in reagents. For example, beef extract lots are reported to contribute to variation in virus recovery rates (Fout et al. 2010). Regardless of the number of recovery controls, these data should be reported along with the virus results for the unknown field samples.

#### 4. ADVANTAGES OF VIRUS SAMPLING

Human enteric viruses are the primary cause of foodborne disease outbreaks, and the food commodity most often implicated is fresh produce (i.e. leafy greens and berries) (Koopmans and Duizer 2004; Scallan et al. 2011). The common assumption is that fresh produce is contaminated with viruses at the point of preparation or service, instead of during production, harvest, and post-harvest steps (Berger et al. 2010; Hall et al. 2012). However, a limited number of studies are available on occurrence of viruses in agricultural water sources (van Zyl et al. 2006; Cheong et al. 2009; Kokkinos et al. 2012; Pachepsky et al. 2011). This knowledge gap stems, in part, from the regulatory emphasis of agricultural water standards that focus on the control and detection of bacteria, not viruses. In the US, the proposed rule for produce safety and standards for the sanitary quality of agricultural water sources is based on *Escherichia coli* density exceedance criteria established by the US EPA for recreational waters (USEPA 2012). These criteria were previously adopted by the California Leafy Greens Marketing Agreement (LGMA) and several other state produce safety programs (LGMA 2013; USFDA 2014).

The variety of agricultural water sources—groundwater wells, ponds, rivers, streams, irrigation ditches, municipal water, reclaimed (treated wastewater)

water, and irrigated liquid manure, combined with the diversity of potential fecal contamination sources, makes it difficult to rely on a single indicator, like *E. coli*, to reflect the fate, transport, and occurrence of all potential pathogens in agricultural water resources. Several criteria define an effective indicator: (1) rapidly measurable, (2) representative of the pathogens of concern, and (3) fate and transport characteristics similar to pathogens (Brookes et al. 2005). Unfortunately, *E. coli* meets only one criterion, rapid measurement. Rarely is *E. coli* fully concordant with pathogenic virus levels (Payment and Franco 1993; Harwood et al. 2005; Payment and Locas 2011). An *E. coli* positive sample suggests the presence of fecal material and indirectly viruses. However, an *E. coli*-negative sample does not necessarily mean viruses are absent. Sampling for viruses provides the definitive advantage of knowing whether viruses are present without the risk of making incorrect inferences based on *E. coli*.

Virus sampling methods offer several other advantages for ensuring the sanitary quality of foods: (1) Methods capable of filtering large sample volumes (e.g., 1000 L) can help account for any spatial and temporal variability of viruses in the water source; (2) Concentrated samples can be stored frozen and analyzed later if necessary; (3) Continuous sampling instead of grab sampling is possible from low turbidity waters using cartridge filter methods like glass wool or hollow fiber ultrafilters; and (4) Some methods are effective in concentrating multiple classes of pathogens (e.g., protozoa, bacteria, and viruses) in one step.

## 5. SUMMARY AND CONCLUSIONS

Based on what has been presented here, it is clear that no single method may universally be recognized as superior or suitable for all monitoring scenarios or contamination events. The efficiency, performance consistency, robustness, complexity of the method, and cost are all factors that must be considered when selecting the appropriate method. Additionally, regardless of the method, performance characteristics must be continuously monitored to ensure recovery efficiency and consistency over time. Finally, there is evidence that applying sampling methods targeted for recovery of viruses and other microorganisms would be advantageous (i.e. when compared to traditional 100 ml grab sample methods for detection of fecal indicator bacteria) in the assessment of the sanitary quality of agricultural water sources.

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