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Concentration and Purification of Viruses by Ultrafiltration – a Short Review

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Abstract

This short review summarizes concentration and purification steps for various viruses in the context of different research applications. The discussed methods have been employed in medical research, monitoring aquatic viruses in the marine environment, and analysis of drinking water and food quality. Furthermore, the persistent threat posed by pathogens with epidemic and pandemic potential has - especially in recent years - brought viruses such as ebola (e.g. EBOV), coronaviruses (e.g. MERS-CoV, SARS-CoV-1 and SARS-CoV-2) and poxviruses (e.g. MPXV) into the spotlight. In these cases, ultrafiltration has broad applicability, from general research, to novel vaccine and treatment development, and disease surveillance. We highlight numerous examples where Vivaspin[®] and Vivaflow[®] ultrafiltration devices have been used in each of these research areas, and include guidance towards selection of the optimal device and molecular weight cut-off (MWCO).

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Introduction

Throughout evolution, viruses have developed various mechanisms to interact with and manipulate the genetic material of their target cells. Based on this, modern molecular biology utilizes viruses in a constantly growing number of applications.¹ They range from controlled genetic transfection of cells to a variety of basic studies in medical science.² In medical studies the strategic focus is on recombinant vaccines and the development of potential vectors for gene therapy.^{3,4}

Besides the great relevance of viruses for medical applications, the assessment of virus type and content is important for the risk assessment of food and drinking water.⁵ Also, the classification of virus content is often of high relevance for the quality control of aquatic biotopes.⁶

During the preparation, handling, or analysis of viruses or virus-like particles (VLPs), a concentration and/or purification step is frequently required.⁵ Typical viruses have a size within the range of about 20 nm up to several hundred nanometers.⁷ Therefore they are ideally suited for retention by ultrafiltration membrane systems and such ultrafilters are widely used in virus research. Specification of the optimal ultrafiltration device typically depends on the particular type of virus, the purpose of ultrafiltration and the subsequent application.

This short review highlights methods for concentration and purification of viruses - especially those which cause illness or disease in humans and other mammals. We summarize the use of ultrafiltration for these processes in the context of medical research, analysis of water and food samples, the purification of marine bacteriophages (virioplankton), and research into new and emerging viruses to understand and monitor transmission, disease spread and novel vaccine development. We also provide guidance for the selection of an ideal performing device with the optimum molecular weight cut-off (MWCO) for each ultrafiltration process.

Applications

Concentration of Viruses in Medical Research

In medical research viruses and VLPs are of major interest, particularly for investigations on infectious viral diseases and for the development of vaccines or antiviral drugs. Moreover, certain VLPs can manipulate genetic material in a directed manner and are used broadly in the development of gene therapy approaches. Additionally, viral vectors are well established as a transfection method for gene transfer into cell lines, for example to manipulate mammalian cells *in vivo* and *in vitro*.

An overview of thematically linked publications using Sartorius ultrafiltration devices for the purification and concentration of viruses and VLPs in the medical context is given in Table 1. Among other applications, Vivaspin® devices were employed for the concentration of adeno-associated virus (AAV) and lentiviral vectors after purification via ion exchange chromatography⁸⁻¹⁰, on blood sera to prepare negative controls from hepatitis C virus (HCV)-positive samples,¹¹ and for the development of a vaccine against human immunodeficiency virus (HIV) and of an antiviral drug against Chikungunya virus.^{12,13} Furthermore, Vivaspin® devices have been used to prepare ebola virus (EBOV) glycoprotein nanoparticles prior to vaccine efficacy testing in mice⁵² and vaccinia virus (VACV) epitope concentration in deimmunization studies towards novel cancer treatments⁵⁹.

Concentration of Viruses from Drinking Water and Food Samples

The guidelines for drinking water quality by the World Health Organization (WHO) describe safety plans to reduce potential risks of various viral infections.¹⁶ They state that, due to the increased resistance of viruses to disinfection methods, an absence of bacterial contamination after disinfection cannot be used as a reliable indicator of the presence or absence of pathogenic viral species in drinking water supplies. Considering this, ultrafiltration can play a vital role towards detecting such viral contamination for research into drinking water quality and food safety.

Prior to ultrafiltration, there is no requirement for samples to be pre-conditioned and concentration efficiency is virtually independent of the chemical properties and structure of the virus.¹⁷ Thus ultrafiltration is very well suited to isolate and concentrate virus particles from water samples and is a valuable aid during the assessment of water quality. Most of the viruses which are found in water and also food samples are of fecal origin. Screening for these viruses - the most frequent being hepatitis A, hepatitis E and norovirus - is therefore crucial to preventing infection outbreaks.¹⁸ Ultrafiltration has been described as the most appropriate method for the recovery of hepatitis A virus from vegetables and other food items.¹⁹ Detection of infectious viruses is mainly done by propagation in cell culture (plaque assay) or the detection of the viral genomes by molecular amplification techniques such as quantitative reverse transcriptase polymerase chain reaction (RT-PCR).²⁰

Table 1

Examples of applications using Vivaspin® and Vivaflow® for virus concentration and purification in medical research.

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultrafiltration Device (MWCO)	Subsequent Step	Ref.
Gene therapy (Adenovirus type 5, VLP, human)	Diafiltration (20 mM Tris saline buffer)	Vivaflow® (100 kDa)	Storage, chromatography on Sartobind STIC (FPLC)	14
Reduction of HCV-induced fibrosis (Hepatitis C Virus; human)	Removal of HCV from human blood serum (Blood serum)	Vivaspin® (30 kDa)	Immunofluorescence assay, fibrosis induction assays	11
Development of a viral entry inhibitor for HIV (HIV, human)	Removal of protein fraction from virus (PBS)	Vivaspin® 20 (1,000 kDa)	Virus inactivation	12
Gene therapy for cancer treatment (adeno-associated virus; rAAV-2, human)	Concentration and purification after expression, Buffer exchange after His tag (FreeStyle 293 Expression Medium (Gibco), serum-free)	Vivaspin® 20 (1,000 kDa)	Titer, ELISA, cell binding assay, apoptosis cell cycle assay	8
System for controlled gene expression in mice brain (Adeno-associated virus, mice)	Concentration of eluate after anion exchange chromatography (elution buffer)	Vivaspin® 20 (100 kDa)	Transduction of mice neurons	9
Efficient gene transfer into the CNS (Lentivirus, human)	Concentration after ion exchange chromatography (PBS)	Vivaspin® (100 kDa)	Real-time PCR and end-point dilution. Transduction of murine neuronal and glial cells <i>in vivo</i>	10
Identification of effective chikungunya antiviral drugs (Chikungunya-Virus, human)	Concentration	Vivaspin® 20 (100 kDa)	Quantification by TCID ₅₀	13
Gene therapy of achromatopsia in mice (Recombinant adeno-associated virus, human virus used in mice)	Concentration (Anion exchange chromatography elution buffer)	Vivaspin® 4 (10 kDa)	Titer determination by dot-blot analysis, subretinal injections	15
Vaccine efficacy testing (EBOV glycoprotein nanoparticles, mouse)	Concentration	Vivaspin® 500 (30 kDa)	Dilution with excipient, coating microneedle patches, BCA assay, SDS PAGE, western blot, quantitative ELISA, vaccination	52
Deimmunization of VACV (recombinant p35 epitopes from VACV, mouse)	Concentration (50 mM Tris-HCl, 150 mM NaCl, pH 7.5)	Vivaspin® Turbo 15 (10 kDa PES)	Immunization	59

Table 2:

Examples of ultrafiltration using Vivaspin® and Vivaflow® for virus concentration from drinking water and food samples.

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultrafiltration Device (MWCO)	Subsequent Step	Ref.
Method for the detection of norovirus genogroup I (Norovirus, human)	Concentration (PBS processed food samples)	Vivaspin® (5 kDa)	RNA extraction for real-time RT-PCR	22
Analysis of viral content in groundwater (A set of pathogenic viruses, potentially human)	Concentration of drinking water sample (Drinking water)	Vivaflow® 200 (10 kDa)	RT-nested PCR and microtiter neutralization test	21
Comparative Analysis of Viral Concentration Methods (Hepatitis A virus, human)	Concentration (0.25 M threonine, 0.3 M NaCl, pH 9.5)	Vivaspin® 20 (100 kDa)	RNA extraction for real-time RT-PCR	19
Analyzing regional gastroenteritis outbreak due to drinking water contamination (Norovirus, Astrovirus, Rotavirus, Enterovirus, Hepatitis A; human)	Concentration (50 mmol/L glycine buffer, 1% beef extract)	Vivaspin® 2	Nucleic acid extraction	23

Concentration of Viruses and Bacteriophages from Marine Samples

In marine biology, the concentration and subsequent analysis of marine bacteriophages (virioplankton) is of major interest. They outnumber the bacterioplankton (their host organisms) by an order of magnitude and thus have an important influence on the whole marine biosphere.²⁴

As described by Wyn-Jones & Sellwood (ref. 17) ultrafiltration can be used to concentrate virus particles in water samples without any prior pretreatment of the sample and it is also

practically independent from the chemical and structural properties of the viruses. Thus, it finds wide use for the analysis of aquatic viruses. For instance, Schroeder *et al.* (ref. 26) were able to determine the diversity and monitor population dynamics of viruses that infect *Emiliana huxleyi*, a globally important photosynthetic plankton. In this study a reusable Vivaflow® 50 unit equipped with a 50 kDa MWCO polyethersulfone (PES) membrane was used to concentrate viruses in sea water samples prior to storage and analysis. For further examples of virus concentration from marine biological samples, see table 3.

Table 3

Examples of ultrafiltration applications using Vivaflow® and Vivaspin® for samples from the marine environment.

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultrafiltration Device (MWCO)	Subsequent Step	Ref.
Assessment of virioplankton diversity (Virioplankton, Plankton)	0.2 µm filtration for clarification, filtrate subjected to 3 kDa filter for concentration (Sea water)	Vivaflow® 200 (0.2 µm and 30 kDa)	Subsequent analysis by DNA separation on Agarose gel	25
Classification of virus (MpRNAV-01B, <i>Micromonas pusilla</i>)	Vivaflow 200: harvest and concentration of whole cell lysate; Vivaspin: washing (removal of CsCl)	Vivaflow® 200, Vivaspin® (30 kDa)	Classification of new virus: genome, proteins, stability, etc.	28
Assessment of genetic diversity in virioplankton (<i>Emiliana huxleyi</i> Bloom virus, Eukaryotic phytoplankton - alga)	After 0.45 µm filtration, concentration 1l to 20 ml (Sea water)	Vivaflow® 50 (50 kDa)	PCR and Denaturing gradient gel electrophoresis	26
Investigation of gene expression during infection (<i>Emiliana huxleyi</i> virus strain 86, Eukaryotic phytoplankton - alga)	Concentration from 5 L to 20 ml (f/2 medium)	Vivaflow® 50 (50 kDa)	CsCl-gradient	27
Study on host genome integration (<i>viroplage mavirus</i> , <i>Cafeteria roenbergensis</i>)	Clarification with 0.2 µm filter and concentration with 100 kDa filter (<i>Cafeteria roenbergensis</i> , f/2 medium)	Vivaflow® 200 (0.2 µm and 100 kDa)	CsCl gradients, electron microscopy	29

Concentration of Coronavirus and its Proteins for General Research

Coronaviruses are spherical, enveloped, RNA based viruses that are typically 80-120 nm in diameter, but in many cases have a diameter outside of this range. Coronavirus genomes are the largest of all RNA viruses which offers a relatively large area of study. Together with high rates of infection, the high chance for future mutations in their large genomes may lead to future human diseases with potential to develop into epidemics and pandemics, such as the recent Middle East Respiratory Syndrome (MERS-CoV), and Severe Acute Respiratory Syndrome 1 (SARS-CoV-1) and 2 (SARS-CoV-2) outbreaks. Hence, further research into the replication, transmission, genome and structure will continue with greater investment of time and funding in the years to come.

A key component to the infection cycle is the coronavirus spike (S) protein, that mediates entry into host cells,

through both attachment and membrane fusion. As such, it is a primary target for the development of novel antiviral drugs and vaccines.

The concentration and purification of both the virions and the spike proteins from cell culture supernatants is often a key requirement to isolate the respective target, prior to structural and functional analysis.

Table 4 highlights several applications where Vivaspin® centrifugal concentrators, or Vivaflow® tangential flow filtration cassettes have been used for the concentration of coronavirus proteins, including the spike protein.

Table 5 provides examples where intact virions or VLPs have been concentrated using the same devices, for various coronaviruses, as well as for a model ebola virus.

Table 4

Examples of concentration and diafiltration of coronavirus proteins using Vivaflow® and Vivaspin®.

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultrafiltration Device (MWCO)	Subsequent Step	Ref.
Neutralization of a SARS-CoV-2 antibody to a functionally conserved receptor binding domain (RBD) on the trimeric spike (S) protein	Buffer exchange of a SARS-CoV-2 RBD protein	Vivaspin® 20 (10 kDa PES)	Protein concentration by UV/Vis and binding affinity by Streptavidin BLI (Sartorius Octet)	30
Investigation of neutralising antibody response on a SARS-CoV-2 spike glycoprotein RBD-SpyVLP (virus-like particle) platform	Concentration of SpyTag-RBD protein construct	Vivaspin® 20 (10 kDa PES)	Purification by SEC	31
Investigation of exosome based vaccines containing coronavirus spike (S) protein, for SARS-CoV-1	Concentration of solubilized spike protein in supernatant	Vivaspin® (10 kDa PES)	Western blot analysis	32
Analyze of the ability to redirect the functionality of the Mouse Hepatitis Coronavirus spike (S) protein to infect human cancer cells	Concentration of cellular receptor protein constructs	Vivaspin®, PES	Western blot analysis	33
Structure determination of Coronavirus SARS-CoV-1 non-structural protein 1 (nsp1)	Concentration of coronavirus nsp1 during purification process	Vivaspin®, PES	Crystalization screening	34
Structure determination of the ADRP domain of Feline Coronavirus (FCoV) non-structural protein 3 (nsp3)	concentration of coronavirus nsp3 during purification process	Vivaspin® (10 kDa PES)	Crystalization screening	35
Investigation into the role of three transmembrane proteases in the activation of SARS-CoV-1 spike (S) protein	Concentration of VLPs from HEK 293T cell culture supernatant	Vivaspin®, PES	Cell-cell fusion assay	36
Cryo-electron microscopy of Human Coronavirus HCoV-NL63 spike glycoprotein trimer that is a potential target for neutralizing antibodies during infection	Concentration of recombinant HCoV-NL63 viruses from clarified Drosophila S2 cell culture supernatant	Vivaflow® (10 kDa PES)	Affinity purification	37

Table 5

Examples of applications using Vivaspin® for coronavirus and ebola viron and VLP sample concentration.

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultrafiltration Device (MWCO)	Subsequent Step	Ref.
Characterisation of phenotypic changes in virus isolates, such as MERS-CoV, that could relate to pandemic potential	Concentration of MERS-CoV virus isolates	Vivaspin® (100 kDa PES)	Quantification using plaque titration Viral RNA sequencing analysis	38
Investigation of antiviral potential of Echinacea purpurea (Echinaforce®) against human coronaviruses; SARS-CoV and MERS-CoV	Concentration of MERS-CoV and SARS-CoV virus dilutions	Vivaspin® 20, PES	Limiting dilution assay (TCID ₅₀)	39
Investigation into inactivation of SARS-CoV-2 through heating and chemical protocols	Concentration and separation of deactivated SARS-CoV-2 from lysis buffer	Vivaspin® 500, PES	Inoculation onto Vero-E6 monolayer	40
Investigation of viral and cellular determinants governing hCoV-EMC entry into host cells	Concentration of SARS-CoV and hCoV-EMC virus like particles (VLPs)	Vivaspin®, PES	Western blot analysis	41, 42
Understanding host cell entry and egress pathways (Ebola model virus: rVSV/EBOV-GP, Vero cells)	Concentration and purification (Iscove's medium)	Vivaspin® 20 (300 kDa PES)	qRT-PCR, plaque assay	53

Concentration of Virions and | or Viral Genomic Material from Wastewater

In humans and birds, coronaviruses may inflict mild to fatal respiratory tract infections, but in other animal groups a range of other diseases may also occur, such as hepatitis and neurological illness⁴². SARS-CoV-2 is the most recent among a string of coronavirus epidemics, which, due to its high infectivity, rates of asymptomatic infection, significant incubation time, our relatively limited knowledge of transmission dynamics and overall lack of global pandemic preparation, developed into a true global pandemic, causing significant impacts on global health, society and economy.

The severity of this pandemic is driving increased research and funding in all associated areas. One area is on the

tracking and epidemiological studies of SARS-CoV-2 infections, with a particular focus on the use of regional wastewater systems, where the compartmentalisation of these systems offers distinct tracking in real time, without the lag for symptom appearance and clinical diagnosis⁴³. In addition, the data collected can be used as a supplemental and low-cost surveillance indicator on the circulation of the virus in a community without the need to screen individuals. Further, it contributes to the tracking of infection prevalence, by adding another epidemic indicator⁴⁴.

RT-PCR is the standard method to test for SARS-CoV-2, but samples typically require concentration and removal of non-coronavirus material prior to testing to ensure optimal results. Ultrafiltration is a successful method for this⁴³, and some examples have been given in Table 6.

Table 6

Examples of ultrafiltration with Vivaflow® and Vivaspin® for virus and viral RNA concentration from wastewater samples.

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultrafiltration Device (MWCO)	Subsequent Step	Ref.
Measurement of SARS-CoV-2 RNA in sewage	Concentration of viral RNA	Vivaspin® (50 kDa PES)	Viral RNA extraction and purification RT-qPCR quantification	43, 44, 45
Benchmarking virus concentration methods for quantification of SARS-CoV-2 in raw wastewater	Concentration of viral RNA	Vivaspin®100 (10 kDa PES)	Viral RNA extraction and purification RT-qPCR quantification	46
Evaluation of two methods to concentrate SARS-CoV-2 from untreated wastewater	Concentration of viral RNA from 40 ml (total) to 700-1000 µl	Vivaspin® (10 kDa)	Viral RNA extraction and RT-qPCR and ddPCR quantification	47
Virus detection in full scale membrane bioreactor (MBR) plant by virus concentration monitoring, inc. Norovirus, Sapovirus and Rotavirus	Concentration of viral particles in effluent	Vivaflow® 50, PES	PEG precipitation Viral RNA quantification	48
Evaluation of membrane bioreactor wastewater virus removal, inc. Norovirus, Sapovirus, Adenovirus	Concentration of effluent from 1L to 40mL	Vivaflow® 50, PES	Nucleic acid extraction RT-PCR quantification	49
Evaluation of membrane bioreactor wastewater Norovirus removal	Concentration of viruses in effluent	Vivaflow® 50, PES	Nucleic acid extraction RT-PCR quantification	50
Evaluation of the association between number of hepatitis E cases in the community and concentration in local sewage	Concentration of viruses in effluent	Vivaflow® 50, PES	Nucleic acid extraction RT-qPCR quantification	51

Concentration of Poxviruses for General Research and Environmental, Animal and Wastewater Sample Analysis

The global effort which culminated in eradication of the variola virus in 1979 was a major achievement in recent history.⁵⁴ However, its potential use as a biological weapon and the 2022 outbreak of the closely related monkeypox virus (MPXV) demonstrate that the poxviruses can still pose a substantial threat to human health.

The current MPXV outbreak - which has now been designated by the WHO as a global health emergency and lacks case links to endemic countries - suggests a possible change to how this virus is transmitted.⁵⁵⁻⁵⁷ Further challenges to controlling this outbreak might also be presented by the long-term stability of orthopoxviruses, and their potential for evolution through recombination, as well as development of resistance against antiviral drugs.⁵⁶⁻⁵⁷ Therefore, it is important to develop a deeper understanding of this virus and support the development of novel control measures, vaccines and treatments.

As for other viruses, ultrafiltration can be used to concentrate and purify poxviruses, and we would suggest higher MWCOs of around 100 - 1,000 kDa, due to their relatively large dimensions of 220 - 450 by 140 - 260 nm, respectively.⁶⁰ Examples where Vivaflow[®] and Vivaspin[®]

have been used in general research, and the analysis of poxvirus content in environmental and animal samples are presented in Table 7.

Finally, to draw parallels with Poliovirus and SARS-CoV-2, MPXV is similarly shed in the faeces of infected individuals prior to symptom onset.⁶¹ This makes wastewater surveillance an attractive technique for monitoring the prevalence and spread of monkeypox infections, as well as the potential emergence of new MPXV variants as the outbreak progresses. Such surveillance methods are already being adapted for and extended to include MPXV in the USA and Thailand.⁶¹⁻⁶³ For this purpose, based on the size and shape of MPXV particles (200-250 nm and brick- or egg-shaped) and genome length (190 kbp)⁵⁵, a 100 or 300 kDa MWCO ultrafiltration membrane might be best suited for optimal particle and DNA recovery, while permitting the removal of low molecular weight interfering substances, during the wastewater sample concentration step.

Table 7

Examples of ultrafiltration with Vivaflow[®] and Vivaspin[®] for concentration of poxviruses and their proteins.

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultrafiltration Device (MWCO)	Subsequent Step	Ref.
Isolation of macromolecular complexes from poxvirus infected cells	Concentration	Vivaspin [®] 500 (10 kDa)	Sucrose density gradient ultracentrifugation, denaturing PAGE	64
Structure determination (Vaccinia virus RNA polymerase)	Concentration and sucrose removal	Vivaspin [®]	cryo-EM	65
Ectromelia virus (ECTV) E163 protein interaction with cell surface glycosaminoglycans (GAGs)	Concentration	Vivaspin [®] 500 (10 kDa)	Flow cytometry	66
Virome characterization of Lake Baikal (<i>Myoviridae</i> , <i>Siphoviridae</i> , <i>Podoviridae</i> , <i>Phycodnaviridae</i> and <i>Poxviridae</i>)	Concentration	Vivaflow [®] 200 and Vivaspin [®] Turbo 15 (50 kDa)	PCR, Sequencing	67
Identification of virus content in pig faeces for aetiology, epidemiology and disease ecology (various mammalian viruses, including Rotavirus, Enterovirus, Sapovirus, Parvovirus, Herpesvirus and Poxvirus)	Concentration	Vivaspin [®] (50 kDa)	CsCl density gradient ultracentrifugation, deep sequencing	68

Conclusion

The concentration and purification of viruses by ultrafiltration is virtually independent of the chemical properties and the structure of the virus particles. As viruses have a size ranging from tens up to several hundred nanometers, they are typically several orders of magnitude bigger than even the largest protein complexes.⁷ Therefore, most viruses are unfailingly retained on membranes with MWCOs up to 1,000 kDa. The exact specifications of the ideal ultrafiltration membranes depend on the purpose of ultrafiltration and the subsequent application(s).

During the preparation of viral vectors for medical studies, a buffer exchange after column purification can be performed with various MWCOs of all sizes.^{8,9,10,15} To separate virus particles from small proteins, a 1,000 kDa cut off has been shown to be effective.¹² For the complete removal of HCV from blood serum a 30 kDa MWCO has been utilized.¹¹ When the assessment of whole virus content is crucial (e.g. food, drinking water or environmental samples) smaller MWCOs (5 – 100 kDa) are used to ensure full recovery of virus particles.^{19,21,22,25-29,67-68}

Ultrafiltration for the concentration of coronavirus species plays an important role in a range of workflows. Perhaps due to the size distribution of viruses and VLPs, there is no standard MWCO used in each study. Typically, for 80-120 nm particles, a 100 kDa MWCO would provide the optimal balance between recovery, removal of interfering substances, speed and shear stresses. However, for the recovery of RNA material, lower MWCOs (10-50 kDa) are recommended to capture a broader range of RNA fragment lengths.

It is a similar situation - albeit with fewer examples in the published literature so far - for the concentration of other infectious viruses. For example, ebola and poxviruses have been concentrated using MWCOs of 300 kDa and 50 kDa, respectively.^{52,67-68} Therefore, until standards are set for each application, it is prudent to test multiple devices and MWCOs when developing an ultrafiltration-based concentration or purification process, especially for new viruses for interest.

Note

Vivaspin® 100 is part of the Vivaspin® product family. Literature published up to c.2022 may reference the use of Vivacell 100, which is a name previously used for the same centrifugal | pressure-driven ultrafilters. When these devices were renamed, there was no change made to fit, form or function, so results collected using Vivacell 100 devices remain valid also for Vivaspin® 100.

Abbreviations

AAV	Adeno-associated virus
CNS	Central nervous system DNA Deoxyribonucleic acid
CoV	Coronavirus
EBOV	Ebola virus
ELISA	Enzyme-linked immunosorbent assay
FPLC	Fast protein liquid chromatography
fCoV	Feline Coronavirus
hCoV	Human Coronavirus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
kDa	Kilodalton (1,000 g per mole)
M	Molarity (mole per litre)
MERS	Middle east respiratory syndrome
mol	Mole
MPXV	Monkeypox virus
MWCO	Molecular weight cut-off
nsp	Nonstructural protein
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PEG	Polyethylene Glycol
PES	Polyethersulfone
RNA	Ribonucleic acid
SARS	Severe acute respiratory syndrome
RBD	Receptor binding domain
BLI	Bio-Layer Interferometry
RT-PCR	Reverse transcriptase-polymerase chain reaction
ddPCR	Droplet digital polymerase chain reaction
TCIDP50	50% Tissue culture infective dose
VLP	Virus-like particle

References

1. Vannucci, L., Lai, M., Chiuppesi, F., Ceccherini-nelli, L. & Pistello, M. Viral vectors : a look back and ahead on gene transfer technology. *New Microb.* 36, 1–22 (2013).
2. Luo, D. & Saltzman, W. M. Synthetic DNA delivery systems. *Nat. Biotechnol.* 8, 33–37 (2000).
3. Ura, T., Okuda, K. & Shimada, M. Developments in Viral Vector-Based Vaccines. *Vaccines* 2, 624–41 (2014).
4. Mingozzi, F. & High, K. A. Therapeutic in vivo gene transfer for genetic disease using AAV: progress and challenges. *Nat Rev Genet* 12, 341–355 (2011).
5. Soule, H., Genoulaz, O., Gratacap-Cavallier, B. Chevallier, P., Liu, J.-X. & Seigneurin, J.-M. Ultrafiltration and reverse transcription-polymerase chain reaction: an efficient process for poliovirus, rotavirus and hepatitis A virus detection in water. *Water Res.* 34, 1063–1067 (2000).
6. Bergh, O., BOrsheim, K. Y., Bratbak, G. & Heldal, M. High abundance of viruses found in aquatic environments. *Nature* 340, 467–468 (1989).
7. Hulo, C. et al. ViralZone: A knowledge resource to understand virus diversity. *Nucleic Acids Res.* 39, 576– 582 (2011).
8. Hagen, S. et al. Modular adeno-associated virus (rAAV) vectors used for cellular virus-directed enzyme prodrug therapy. *Sci. Rep.* 4, 3759 (2014).
9. Schindler, S. E. et al. Photo-activatable Cre recombinase regulates gene expression in vivo. *Sci. Rep.* 5, 13627 (2015).
10. Scherr, M. et al. Efficient gene transfer into the CNS by lentiviral vectors purified by anion exchange chromatography. *Gene Ther.* 9, 1708–1714 (2002).
11. Granato, M. et al. HCV derived from sera of HCV-infected patients induces pro-fibrotic effects in human primary fibroblasts by activating GLI2. *Sci. Rep.* 6, 30649 (2016).
12. Martin, L. et al. Rational design of a CD4 mimic that inhibits HIV-1 entry and exposes cryptic neutralization epitopes. *Nat. Biotechnol.* 21, 71–76 (2003).
13. Karlas, A. et al. A human genome-wide loss-of-function screen identifies effective chikungunya antiviral drugs. *Nat. Commun.* 7, 11320 (2016).
14. Nestola, P. et al. Rational development of two flowthrough purification strategies for adenovirus type 5 and retro virus-like particles. *J. Chromatogr. A* 1426, 91–101 (2015).
15. Carvalho, L. S. et al. Long-term and age-dependent restoration of visual function in a mouse model of CNGB3-associated achromatopsia following gene therapy. *Hum. Mol. Genet.* 20, 3161–3175 (2011).
16. Guidelines for drinking-water quality - 4th ed. World Health Organization 2011.
17. Wyn-Jones, a P. & Sellwood, J. Enteric viruses in the aquatic environment. *J. Appl. Microbiol.* 91, 945–962 (2001).
18. Botzenhart, K. Viren im Trinkwasser. *Bundesgesundheitsblatt - Gesundheitsforsch. - Gesundheitsschutz* 50, 296–301 (2007).
19. Lee, K. B., Lee, H., Ha, S. D., Cheon, D. S. & Choi, C. Comparative analysis of viral concentration methods for detecting the HAV genome using real-time RT-PCR amplification. *Food Env. Virol.* 4, 68–72 (2012).
20. Bosch, A. et al. Analytical Methods for Virus Detection in Water and Food. *Food Anal. Methods* 4, 4–12 (2011).
21. Masciopinto, C. et al. Unsafe tap water in households supplied from groundwater in the Salento Region of Southern Italy. *J. Water Health* 5, 129–148 (2007).
22. Dreier, J., Störmer, M., Mäde, D., Burkhardt, S. & Kleesiek, K. Enhanced reverse transcription-PCR assay for detection of norovirus genogroup I. *J. Clin. Microbiol.* 44, 2714–2720 (2006).
23. Maunula, L. et al. Enteric Viruses in a Large Waterborne Outbreak of Acute Gastroenteritis in Finland. *Food Environ. Virol.* 1, 31–36 (2009).
24. Wommack, K. E. & Colwell, R. R. Virioplankton: viruses in aquatic ecosystems. *Microbiol. Mol. Biol. Rev.* 64, 69–114 (2000).
25. Parada, V., Baudoux, A.-C., Sintes, E., Weinbauer, M. G. & Herndl, G. J. Dynamics and diversity of newly produced virioplankton in the North Sea. *ISME J.* 2, 924–936 (2008).
26. Schroeder, D. C., Oke, J., Hall, M., Malin, G. & Wilson, W. H. Virus Succession Observed during an *Emiliania huxleyi* Bloom Virus. *Appl. Environ. Microbiol.* 69, 2484– 2490 (2003).
27. Allen, M. J. et al. Locus-Specific Gene Expression Pattern Suggests a Unique Propagation Strategy for a Giant Algal Virus. *J. Virol.* 80, 7699–7705 (2006).
28. Brussaard, C. P. D., Noordeloos, A. A. M., Sandaa, R. A., Heldal, M. & Bratbak, G. Discovery of a dsRNA virus infecting the marine photosynthetic protist *Micromonas pusilla*. *Virology* 319, 280–291 (2004).
29. Fischer, M. G. & Hackl, T. Host genome integration and giant virus-induced reactivation of the virophage mavirus. *Nature* 540, 288–291 (2016).
30. Liu, H. et al. Cross-Neutralization of a SARS-CoV-2 Antibody to a Functionally Conserved Site Is Mediated by Avidity. *Immunity* 53, 1272–1280 (2020)
31. Tan, T.K. et al. A COVID-19 vaccine candidate using SpyCatcher multimerization of the SARS-CoV-2 spike protein receptor-binding domain induces potent neutralizing antibody responses. *Nature Communications* 12:542 (2021)

32. Kuate, S. et al. Exosomal vaccines containing the S protein of the SARS coronavirus induce high levels of neutralizing antibodies. *Virology* 362, 26-37 (2007).
33. Würdinger, T. et al. Soluble receptor-mediated targeting of mouse hepatitis coronavirus to the human epidermal growth factor receptor. *J. Virology* 79, 15314-15322 (2005)
34. Jansson, A. M. Structure of Alphacoronavirus transmissible gastroenteritis virus nsp1 has implications for coronavirus nsp1 function and evolution. *J Virology* 87, 2949-2955 (2013),
35. Justyna, A. et al. Structure of the X (ADRP) domain of nsp3 from feline coronavirus. *Bio. Crystallography* 65, 1292-1300 (2009).
36. Bertram, S. et al. Cleavage and activation of the severe acute respiratory syndrome coronavirus spike protein by human airway trypsin-like protease. *J Virology* 85, 13363-13372 (2011).
37. Walls, A. C. et al. Glycan shield and epitope masking of a coronavirus spike protein observed by cryo-electron microscopy. *Nature Str & M. Biology* 23 (2016).
38. Schroeder, S. et al. Functional comparison of MERES-coronavirus lineages reveals increased zoonotic potential of the recombinant lineage 5. Pending publication.
39. Signer, J. In vitro antiviral activity of Echinaforce®, an Echinacea purpurea preparation, against common cold coronavirus 229E and highly pathogenic MERS-CoV and SARS-CoV. Pending publication
40. Pastorino, B. et al. Evaluation of heating and chemical protocols for inactivating SARS-CoV-2. Pending publication
41. Gierer, S. et al. The spike protein of the emerging betacoronavirus EMC uses a novel coronavirus receptor for entry, can be activated by TMPRSS2, and is targeted by neutralising antibodies. *J. Virology* 87, 5502-5511 (2013).
42. Masters, P. S. The Molecular Biology of Coronaviruses. *Adv. Virus Research* 66, 193-292 (2006)
43. Larsen, D. A, Wigginton K.R. Tracking COVID-19 with Wastewater. *Nature Biotechnology* 38, 1151-1153 (2020).
44. Trottier, J. et al. Post-lockdown detection of SARS-CoV-2 RNA in the in the wastewater of Montpellier, France. *One Health* 10 (2020)
45. Hokkaido University. SARS-CoV-2 RNA Detected in Untreated Wastewater from Louisiana. *ScienceDaily*. (26 Aug 2020)
46. Jafferalli, M. H. et al. Benchmarking virus concentration methods for quantification of SARS-CoV-2 in raw wastewater. *Science of the Total Environment* 10, 755, (2021)
47. Dumke, R. et al. Evaluation of Two Methods to Concentrate SARS-CoV-2 from Untreated Wastewater. *Pathogens* 195 (2021)
48. Takayuki, M. et al. Virus type-specific removal in a full-scale membrane bioreactor treatment process. *Food and Env. Virology* 10, 176-186 (2017).
49. Sima, L. C. et al. Calicivirus removal in a membrane bioreactor wastewater treatment plant. *Applied and Inv. Microbiology* 77, 5170-5177 (2011).
50. Schaeffer, J. et al. Improving the efficacy of sewage treatment decreases norovirus contamination in oysters. *Int. J. of Food Microbiology* 286, 1-5 (2018).
51. Takayuki, M. et al. Detection of hepatitis E virus in sewage after an outbreak on a French island. *Food and Env. Virology* 8, 194-100 (2016).
52. Liu, Y. et al. Intradermal immunization by Ebola virus GP subunit vaccines using microneedle patches protects mice against lethal EBOV challenge. *Scientific Reports* 8, 11193 (2018).
53. Acciani, M. D. et al. Ebola virus requires phosphatidylserine scrambling activity for efficient budding and optimal infectivity. *Proceedings* 50, 35 (2020).
54. Henderson, D. A. Smallpox Eradication. *Public Health Reports* 95, 422-426 (1980).
55. WHO Director-General's Statement at the Press Conference Following IHR Emergency Committee Regarding the Multi-Country Outbreak of Monkeypox. 23 July 2022. Available at: www.who.int (Accessed: 26 July 2022).
56. Quarleri, J. and Delpino, M. V. Monkeypox: Considerations for the Understanding and Containment of the Current Outbreak in Non-Endemic Countries. *GeroScience*, pre-proof (2022).
57. Xiang, Y. and White, A. Monkeypox virus emerges from the shadow of its more infamous cousin: family biology matters. *Emerging Microbes and Infections* 11, 1768-77 (2022).
58. Luo, Q. and Han, J. Preparedness for a Monkeypox Outbreak. *Infectious Medicine* 22, pre-proof (2022).
59. Ramos, R. N. et al. Pseudocowpox Virus, A Novel Vector to Enhance the Therapeutic Efficacy of Antitumor Vaccination. *Clinical and Translational Immunology* (2022).
60. Petersen, B. W. and Damon, I. K. in *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases* 132, 1809-1817 (2020).
61. Thai Researchers Test Wastewater to Track Spread of Monkeypox. 28 July 2022. Available at: www.theguardian.com (Accessed: 29 July 2022).
62. Darrow, M. Waste Water COVID Detection Efforts Extended to Look for Monkeypox. 28 July 2022. Available at: www.cbsnews.com (Accessed: 29 July 2022).
63. Williams, R. The Download: Monkeypox Detection in Wastewater. 25 July 2022. Available at: www.technologyreview.com (Accessed: 29 July 2022).

64. Bartuli, J. et al. A Generic Protocol for the Affinity-Purification of Native Macromolecular Complexes from Poxvirus-Infected Cells. *STAR Protocols* 3, 101116 (2022).
65. Grimm, C. et al. Structural Basis of Poxvirus Transcription: Vaccinia RNA Polymerase Complexes. *Cell* 179, 1537-50 (2019).
66. Heidarieh, H. and Alcamí, A. Mechanism of Action of the Viral Chemokine-Binding Protein E163 from Ectromelia Virus. *JBC* 293, 17418-29 (2018).
67. Potapov, S. A. et al. Metagenomic Analysis of Virioplankton from the Pelagic Zone of Lake Baikal. *Viruses* 11, 991 (2019).
68. Sachsenröder, J. et al. Simultaneous Identification of DNA and RNA Viruses Present in Pig Faeces Using Process-Controlled Deep Sequencing. *PLoS One* 7, e34631 (2012).

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