SARTORIUS

Application Guide

February 18, 2021

Keywords or phrases:
Ultrafiltration, Virus Purification, Antiviral Drugs, Vaccines, Gene Therapy, Bacteriophage, Coronavirus, SARS-CoV-2, Wastewater

Concentration and Purification of Viruses by using Ultrafiltration, Incl. Coronavirus – a Short Review

Hannes Landmann[2], Kristin Menzel[2], Adam Green[1*], Klaus Schöne[1]

[1] Sartorius Lab Instruments GmbH & Co. KG, Otto-Brenner-Straße 20, 37079 Göttingen, Germany

[2] Sartorius Stedim Biotech GmbH, August-Spindler-Straße 11, 37079 Göttingen, Germany

* Correspondence: adam.green@sartorius.com

Abstract

This short review highlights concentration and purification steps of various viruses in the context of different research applications. The discussed methods find application in medical research, marine biology as well as in research concerning drinking water and food quality. You will find a guidance for the selection of an ideal performing ultrafiltration device with the optimum molecular weight cut-off (MWCO) for typical concentration applications.

With the emergence of the COVID-19 global pandemic, the large Coronavirus family of viruses has come to the forefront of immunology, epidemiology and vaccinology studies. Investigations into general Coronavirus virology, Coronavirus spike proteins, capture of Coronavirus virons and free RNA from water streams, are three core examples of focus points for study in the scientific community, as such we provide some examples of concentration and purification steps in these Coronavirus research workflows also (found in tables 5 and 6).

Find out more at:
https://www.sartorius.com/en/products/lab-filtration-purification/ultrafiltration-devices

Introduction

Evolutionary, viruses developed various mechanisms to interact 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 different basic studies in medical science.[2] In medical studies the strategic focus is on recombinant vaccines and on 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 the retention on ultrafiltration membrane systems and such ultrafilters are widely used in basic virus research. The specifications of such ultrafiltration devices depend on the particular type of virus and the purpose of the subsequent application.

This short review highlights methods for the purification of various mammalian viruses for basic medical research. Also, the concentration of pathogenic viruses from water and food samples and the purification of marine bacteriophages (virioplankton) are highlighted. It will also give guidance for the selection of an ideal performing device with the optimum molecular weight cut-off (MWCO) for the user specified ultrafiltration process.

Concentration of mammalian 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 genetic therapy approaches. Additionally, viral vectors are well established as a transfection method for gene transfer to cell lines e. g. 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 chromatography[8–10] on blood sera to prepare blank samples from hepatitis C virus (HCV)-positive blood sera,[11] for the development of a vaccine against human immunodeficiency virus (HIV) and of an antiviral drug against Chikungunya virus.[12,13]

Concentration of viruses from drinking water and food samples
The guidelines for drinking-water quality by the world health organization describe safety plans to reduce potential risks from various virus infections.[16] It states 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 | absence of pathogenic viral species in drinking water supplies. Considering this, ultrafiltration can play a vital role in detecting such viral contaminations for the research on drinking water quality and food safety.

For an ultrafiltration step, the water sample does not have to be pre-conditioned and its efficacy in concentrating the virus is virtually independent of the chemical properties and the 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 is crucial to prevent infections. The most frequent ones are hepatitis A, hepatitis E and norovirus.[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

Summarized examples of applications with Vivaspin® and Vivaflow® for of viruses 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 membrane absorber (FPLC)	14
Reduction of HCV-induced fibrosis (Hepatitis C Virus; human)	Removal of HCV from human blood serum (Blood serum)	Vivaspin® (30 kDa)	Preparation of negative control (from positive sample) for 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)	Quantification via real-time PCR and end-point dilution. Transduction of murine neuronal and glial cells *in vivo*	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

Table 2:

Summarized examples of ultrafiltration application with Vivaspin® and Vivaflow® with viruses 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)	Qualitative analysis (enterovirus) by 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
Analysis of regional outbreak of gastroenteritis due to drinking water contamination (Norovirus, Astrovirus, Rotavirus, Enterovirus, Hepatitis A virus; human)	Concentration (50 mmol/L glycine buffer, 1% beef extract)	Vivaspin® 2	Nucleic acid extraction	23

Concentration of viruses and bacteriophages from marine biological 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 *Emiliania huxleyi*, a globally important form of photosynthetic plankton. In this study a reusable Vivaflow® 50 unit equipped with a polyethersulfone (PES) membrane with MWCO of 50 kDa 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

Summarized examples of ultrafiltration applications with Sartorius Vivaflow® and Vivaspin® of samples from marine biology

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, *Micromonas pusilla*)	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 (*Emiliania huxleyi* 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 (*Emiliania huxleyi* 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 (*virophage mavirus, Cafeteria roenbergensis*)	Clarification with 0.2 µm filter and concentration with 100 kDa filter (*Cafeteria roenbergensis*, f/2 medium)	Vivaflow® 200 (0.2 µm and 100 kDa)	CsCl gradients, electron microscopy	29

Concentration of Coronavirus for general research and protein research (spike protein)

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. Correlatingly the potential for future mutations in this large genome may lead to future human diseases that may evolve into epidemics and pandemics, such as the previous Middle East Respiratory Syndrome (MERS-CoV), and Severe Acute Respiratory Syndrome 1 (SARS-CoV-1) and 2 (SARS-CoV-2). 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 virons and the spike proteins from cell culture and supernatants is often a key requirement to isolate the respective target, prior to structural, functional analysis and binding assays, etc.

Table 4 highlights several applications where Vivaspin® centrifugal concentrators, or Vivaflow® tangential flow filtration cassettes have been used for the concentration or Coronavirus proteins, including the spike protein. References are also provided to direct readers for detailed reading.

Table 5 provides examples of concentration of intact virons, or Coronavirus virus like particles (VLPs), with the same devices.

Table 4

Summarized examples of ultrafiltration applications with Sartorius Vivaflow® and Vivaspin® of coronavirus protein samples.

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®, PES (10 kDa)	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® 20PES (10 kDa)	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®, PES (10 kDa)	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®, PES (10 kDa)	Crystalization screening	35
Investigation into the role of three transmembrane proteases in the activation of SARS-CoV-1 spike (S) protein	Concentration of VLPSs 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 potetial target for neutralizing antibodies during infection	Concentration of recombinant HCoV-NL63 viruses from clarified Drosophila S2 cell culture supernatant	Vivaflow®, PES (10 kDa)	Affinity purification	37

Table 5

Summarized examples of ultrafiltration applications with Sartorius Vivaflow® and Vivaspin® of coronavirus viron and VLP samples

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultrafiltration Device (MWCO)	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®, PES (100 kDa)	Vivaspin®, PES (100 kDa)	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	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	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	Vivaspin®, PES	Western blot analysis	41, 42

Concentration and capture of virons and / or viral RNA in 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 illness[42]. SARS-CoV-2 is the most recent among a string of Coronavirus epidemics, which early indications suggest that 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, has evolved into a true global pandemic and has caused significant impact on global health, society and economy.

The severity of this pandemic is driving increased research and funding in all associated areas. Once area is on the tracking and epidemiological studies of SARS-CoV-2 infections. One area of focus is in 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 diagnosis[43]. 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 indicator[44].

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 this[43], and some examples have been given in Table 6.

Table 6

Summarized examples of ultrafiltration applications with Sartorius Vivaflow® and Vivaspin® of virus and viral RNA in 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®, PES (50 kDa)	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	Vivacell®, PES (10 kDa)	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

Conclusion

The purification of virus by ultrafiltration is virtually independent of the chemical properties and the structure of the virus particles. As viruses have a size within the range of about 20 nm up to several hundred nanometers, they are typically several orders of magnitude bigger than even the biggest protein complexes.[7] Therefore, most viruses are unfailingly retained on membranes with large MWCOs of up to 1,000 kDa. The exact specifications of the ideal ultrafiltration membranes depend on the purpose of the subsequent application.

Ultrafiltration for the concentration of Coronavirus species plays and important role in a range of workflows. Perhaps due to the size distribution of viruses and VLPs, the exact MWCO used is not standard across each study. Although typically, for 80-120 nm particles the 100 kDa MWCO would provide the optimal balance between recovery, removal of interfering substances, speed and shear stresses. Whereas for the recovery of RNA material, lower MWCOs (10-50 kDa) are recommended to capture a greater range of RNA chain lengths. However, until further standardisation is confirmed for each application, it is prudent to test specific devices before implementing into procedures.

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 work.[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 marine water samples) smaller MWCOs (5 – 100 kDa) are used to ensure full recovery of virus particles.[19,21,22,25-29]

Abbreviations

AAV	Adeno-associated virus
CNS	Central nervous system DNA Deoxyribonucleic acid
CoV	Coronavirus
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 (1000 g per mole)
M	Molarity (mole per litre)
MERS	Middle east respiratory syndrome
mol	Mole
MWCO	Molecular weight cut-off
nsp	Nonstructural protein
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).

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further contacts, visit
www.sartorius.com

Status: 03 | 2021