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Viral Safety

Are You Doing Everything to Mitigate Your Risk?

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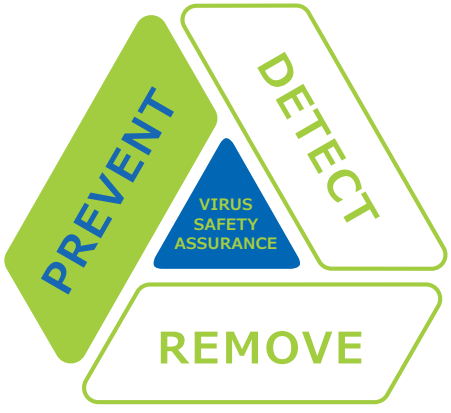

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Viral Safety

Are You Doing Everything to Mitigate Your Risk?

CONTENTS

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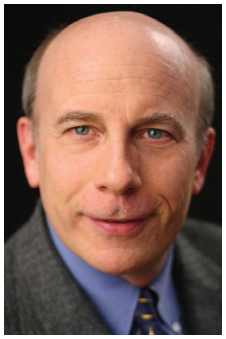
Welcome


Darren Verlenden


John Sterling

Dear GEN Readers,

Viral safety is a constant concern for all biopharmaceuticals as a contamination can cause significant regulatory concern and business disruption.

A multilayered approach to virus safety across the full production landscape involves treating raw materials to prevent virus from entering the upstream process, testing intermediates to detect virus, and implementing purification and filtration technologies to remove virus downstream.

Recognizing there is no single solution that works for every process, experts from MilliporeSigma are hoping to stimulate discussion by highlighting different aspects of viral safety for well-established platforms and newer virus-based therapies. We hope you find these perspectives informative and look forward to working with you to solve the toughest viral safety challenges.

Darren Verlenden
Vice President, Bioprocessing,
MilliporeSigma

John Sterling
Editor-in-Chief, GEN
jsterling@GENengnews.com

GENengnews.com

MilliporeSigma

GEN
Genetic Engineering & Biotechnology News

Advances in Upstream Technologies Reduce Viral-Contamination Risks

Multilayered Approach Includes Virus-Resistant CHO Cell Lines, Advanced Filtration Technologies, and Careful Raw Material Selection

K. John Morrow, Jr. Ph.D.

Viral contamination in biopharmaceutical processes is the enemy within—creating major problems for the biomanufacturer and significant potential risk for patient safety. Contamination constitutes a major challenge to the biopharmaceutical industry, and is now being vigorously attacked on multiple fronts.

On the upstream end, the CHO cell line that has been the workhorse of the biologics industry for decades is being reinvented to improve the line's robustness and performance. At the same time, improvements in treatment and testing of media components have dramatically lowered the risk of introducing virus contamination to the downstream process. This multilayered approach has dramatically lowered the risk of catastrophic failures.

Engineering Better Cell Lines

According to Joaquina X. Mascarenhas, Ph.D., team lead, host cell-line engineering at MilliporeSigma, CHO cell lines are the preferred host-expression system for many therapeutic proteins, such as antibodies, hormones, and blood factors. The team's focus is on manipulating sublines of CHO cells to enable them to work faster and more effectively for biomanufacturing purposes.

"We are moving toward next-generation expression systems for the manufacture of recombinant therapeutic proteins and vaccines with superior attributes, such as ➜

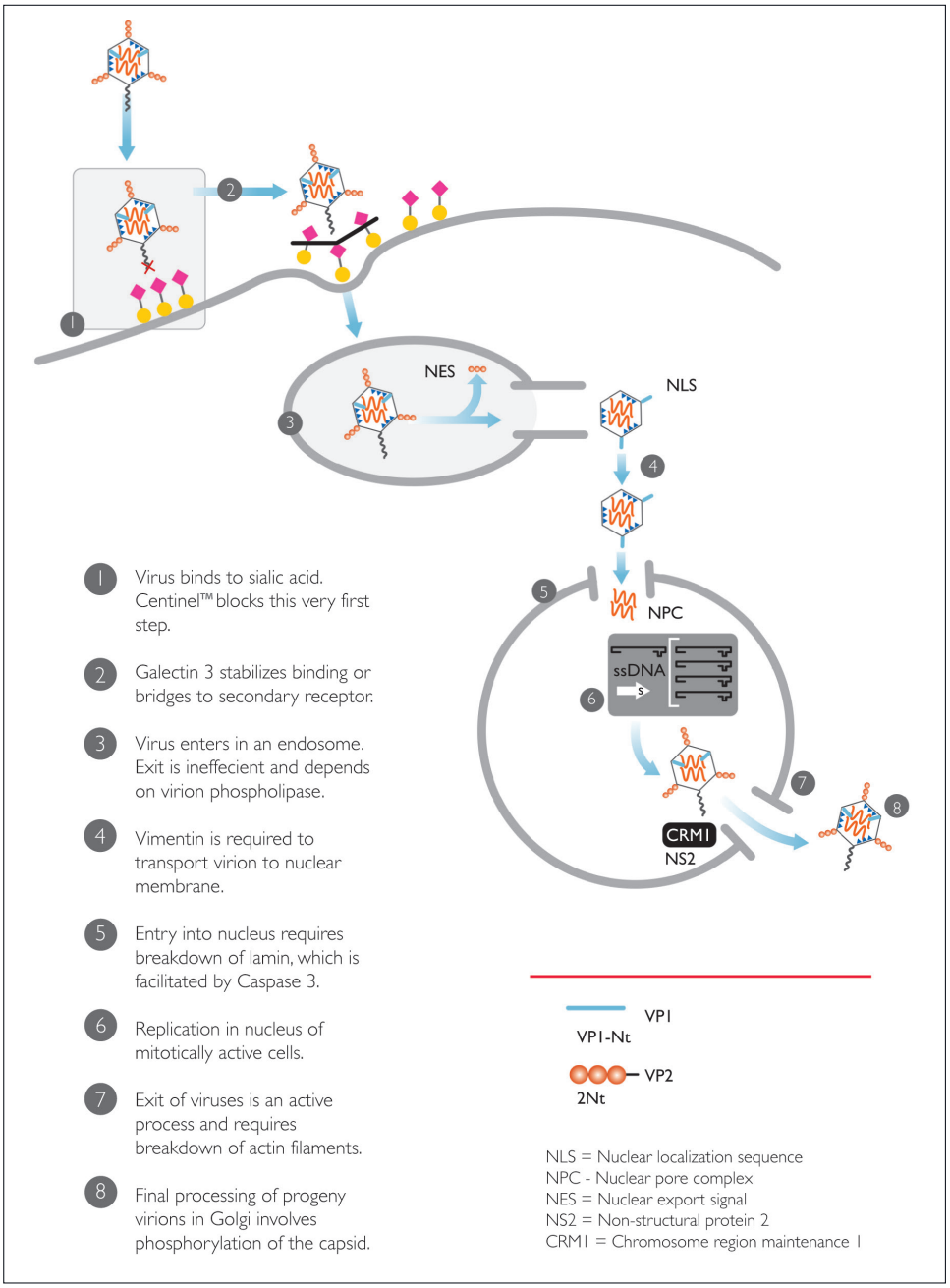
Upstream suite in a mAb manufacturing plant. A major component of a virus safety strategy is preventing viruses from entering the upstream process.

Advances in Upstream Technologies Reduce Viral-Contamination Risks

Continued from page 5

improved product quality, higher productivity, and shorter development timelines," asserted Dr. Mascarenhas.

Dr. Mascarenhas led a team of scientists that developed a genetically engineered CHO host cell line refractory to viral contamination. She and her colleagues have employed genetic engineering to make the CHO line resistant to the parvovirus, minute virus of mice (MVM). This was accomplished by modifying the major receptors used by the virus to enter the cell. "Our goal was to ensure that the cell lines were inherently resistant to MVM contamination, as this is the cell's last line of defense," she explained.



The mechanism of action for viral resistance in a Centinel Intelligent Virus Defense™ modified CHO line.

In a peer-reviewed study, the group reported that having recognized the role of sialic acid in mediating virus entry into the cell, they developed a cell line with modified sialic acid glycan structures which lacked the ability to bind MVM. The development of this cell line has resulted in the first commercially available gene-editing approach for the creation of MVM-resistant CHO cells: Centinel Intelligent Virus Defense™ technology.

Dr. Mascarenhas explained that while a majority of the known viral contaminants in CHO lines were introduced through contaminated animal-derived components, MVM contamination continues to be a threat, even in chemically defined processes. Although it is impossible to completely remove the risk of viral contamination, using CHO cells that are resistant to one of the biggest threats to the industry should greatly enhance even the most comprehensive viral-mitigation programs in use.

Ensuring Product Quality

"Companies need to implement a multilayered approach for their viral risk mitigation strategy," explained Kevin Kayser Ph.D., head of upstream R&D at MilliporeSigma. "For example, we are constantly evaluating our raw materials used in cell culture-media manufacturing. We establish robust procedures for the selection and approval of vendors and raw materials to aid in this risk mitigation."

According to Dr. Kayser, viral contaminants ordinarily enter the process train from various sources, such as cells (endogenous), raw materials (serum), or manufacturing processes.

"Companies cannot detect viral contamination at the level of one viral particle per liter, yet it is known that this is sufficient to infect a biologics manufacturing run, causing contamination and loss of the product," Dr. Kayser explained. "The industry's sampling and detection methods aren't capable of this low-level detection. We take the risk very seriously, and use a variety of strategies to mitigate against viral contamination risk."

Technologies such as high temperature short time (HTST) or virus filtration designed specifically for upstream media components provide alternatives for upstream viral safety.

In considering the future direction of the cell lines used in biologics production, Dr. Kayser believes that modified and engineered versions of the CHO line will be the protein synthetic factory of choice for the foreseeable future. "CHO cells have proven to be a very successful tool in protein drug production," he stated. "Yes, there are alternatives that people are thinking about, but certification of a new cell line is a costly and time-consuming process and there would have to be a powerful incentive in order to embark upon this course of action." Virus-risk reduction may be one of those powerful incentives.

> To date there have been no instances of disease-causing organisms transmitted through recombinant DNA-derived therapeutics.

Dealing with adventitious agents in culture systems is a work in progress. "We are always trying to find process changes that will save costs and ultimately lower the economics of drug production," Dr. Kayser asserted. "The field is constantly evolving, and new adventitious-agent threats are always on the horizon. I foresee ➜

Survey by the CAACB

"Viral contamination events may cause significant impact on a bioproduction facility," according to Paul W. Barone, Ph.D., director of the Consortium on Adventitious Agent Contamination in Biomanufacturing (CAACB) at MIT. "For this reason, our consortium focuses on collective learning to prevent these events in biopharmaceutical manufacturing."

The CAACB provides a forum for networking, sharing experiences, collaborating on projects and promoting new technology to mitigate the risk of a contamination. According to Dr. Barone, viral contamination of mammalian cell lines is hardly an academic exercise. From a survey performed by the consortium, the group is aware of 26 contamination events, the majority of which occurred in the CHO cell line.

"Given its ubiquitous presence in bioproduction protocols, this is not surprising. The consequences of these events affect all aspects of a company's production facility, and can be catastrophic, costing millions of dollars and causing shutdowns for months," he explained. "Moreover, contamination events have been reported in all stages of development, from preclinical through commercial, with the cost of their mitigation rising astronomically in the late stages."

It is noteworthy that for the CHO cell cultures, even though all virus contaminants were suspected to have come from culture-media components, testing did not eliminate the risk of contamination—an observation highlighting the difficulty of detecting very low levels of contamination. In at least one instance, a non-animal raw material was directly identified as the source of the virus contamination.

"As a way to reduce the risk from different media components, the consortium has evaluated the effectiveness of different technologies to remove or inactivate viruses in media," Dr. Barone explained. "UV-C irradiation, physical separation using filtering devices, and heat treatment were all, in general, found to be effective."

On the other hand, for human and primate cell culture, the source of virus contaminants was attributed to human sources.

Dr. Barone said he is proud of the bioindustry's long-term record.

"In 30-plus years of cell culture-based biopharmaceutical manufacturing, no recombinant DNA-derived product has been shown to transmit a viral safety problem," he pointed out. ■

Advances in Upstream Technologies Reduce Viral-Contamination Risks

Continued from page 7

there will be advances introduced in the near term that companies will employ to control viral and other contaminating agents."

Focus on Biosafety Issues

"In advising customers on production issues, we employ what we refer to as the biosafety triangle: prevention, detection and removal," explained Darren Verlenden, Vice President of Bioprocessing at MilliporeSigma. "Because we monitor the guidelines of regulatory bodies worldwide, we can assist customers in the interpretation of directives established by different countries."

Verlenden expanded on the comments of the other interviewees. "We have been developing this pathway over the last few years," he noted. "We recognize that newly emerging companies might not have the level of resources of more established clients, so we have to tailor our responses to match the client's individual situation. The approach is really holistic in which we assist the customer to understand and quantify risk. We help evaluate different risk mitigation approaches dependent on customers risk perception."

To prevent virus entry into production processes, Verlenden stresses that it is essential to carefully monitor extraneous input, as contaminants are invariably introduced through outside sources. "This means we have to look very closely at suppliers, and be assured that they have a long-standing pattern of mature quality control, and tight warehouse management, which is especially critical." Advances in preventing virus entry into upstream purification increase confidence for viral safety in your purified product.

The Expanding Power of New Technologies

Increasing awareness of the risks associated with upstream vial contamination has fueled development of products and technologies to meet the needs of today's biomanufacturing processes. New filtration technology, capable of removing virus, mycoplasma, and bacteria, enables efficient processing of cell culture media before entry to the bioreactor. Importantly, these novel filters don't change the properties of the cell culture media and provide an easy-to-implement solution that can be integrated into upstream processes.

Colette Côté, Ph.D., expanded the discussion on recent technologies describing next-generation sequencing (NGS) or massively parallel sequencing (MPS) in detecting viral contaminants. "It has proven to be a powerful tool in the maintenance of sterility from early development to the final product," she explained.

NGS, and the power of computers to analyze and search bioinformatics databases, is a robust new tool to complement more traditional cell-based methods for virus detection. It is not limited to virus detection, but will detect a broad range of adventitious agents such as bacteria or mycoplasma without assumptions of the nature of the agent.

"My colleague, Dr. Arifa Khan at the U.S. FDA, provided an interesting example" pointed out Dr. Côté. "They used NGS to identify a rhabdovirus contaminant in the sf9 cell line from *Spodoptera frugiperda*, an important cell line used in biotechnology in protein production protocols."

Because sf9 is an insect cell line, it would be expected to present fewer safety issues during protein production than mammalian cells. Identification of this virus contaminant by NGS was critical to risk mitigation in the sf9 system: "We find that our bioinformatics capability enables us to answer questions asked by the entire industry in a much shorter time frame than many of the traditional assays," said Dr. Côté.

It is important to note that NGS or MPS is a so-called "reactionary tool." Dr. Côté described it in this fashion: "Say your bioreactor just crashed and you want to know why, without making assumptions as to the cause. You can analyze your material in an unbiased fashion, quickly and in an affordable manner."

To facilitate sequence analysis, Dr. Côté described an essential one-step identification tool, referred to by the acronym BLAST. Developed by the National Center for Biotechnology Information, the Basic Local Alignment Search Tool is remotely accessible and accepts sequence inputs, comparing them to the local database of sequences on record. Widely used in the virus-identification process, it has proven invaluable in rapid identification protocols for viral contaminants. The software also includes tools for identification of mutational changes in target sequences that may have been introduced during the PCR amplification process.

Conclusions

While workers in the field recognize that total elimination of viral contaminants at the upstream end of the process is not possible, it is clear that new technologies have lowered the risk to manageable levels. Whereas in the past there have been instances of virus transmission to patients through contaminated plasma and blood samples, subsequent improvements to screening procedures and downstream purification operations have reduced this risk.

To date, there have been no instances of disease-causing organisms transmitted through recombinant DNA-derived therapeutics; a strong endorsement of the step-by-step improvements that we have seen over the years within the industry, and a positive harbinger for the future.

Viral Safety in Monoclonal Antibody Manufacturing

Various Technologies to Prevent, Detect, and Remove Virus Contamination

Angelo DePalma, Ph.D.

The significance of viral safety is apparent throughout the biopharmaceutical production process. The ultimate goal is to protect patients from pathogenic viruses, and biopharmaceutical manufacturers need to demonstrate viral safety and validate viral clearance capability of the manufacturing process before market approval.

Viral safety is critically important in both upstream and downstream processes. Factors to consider in upstream processes include: choice of expression system, degree and type of genetic manipulation of those cells or organisms, and how the cell culture is run. All approaches are selected to efficiently produce the biotherapeutic product while minimizing the possibility of virus entry into the system. In downstream purification, virus removal or inactivation is accomplished by a combination of orthogonal or complementary approaches that include chromatography, chemical inactivation, and filtration.

For this article, we turn to four experts from MilliporeSigma, all with unique perspectives on the various technologies that assure that biotherapeutic protein products not only comply with requirements for cGMPs and expectations of regulators, but ultimately provide the highest level of patient safety.

Adventitious viruses can enter the production processes from multiple different routes: from cells, raw materials, personnel, or the environment. In addition, mammalian expression cells contain endogenous viruses. "These viruses are known, quantifiable, and represent fixed-input virus levels that establish a baseline demand for removal or inactivation," explains David Beattie, Ph.D., Head of Bioprocessing R&D, MilliporeSigma. Virus titers vary according to cell type, transfection methods, and expressed protein. For example, the NS0 cell line expresses higher virus titers than the CHO cell line, as do expression systems that produce cytokines. Thus, the inherent variability and uniqueness in addressing viral safety, according to Dr. Beattie, is "having a variable level of input virus that defines a baseline →

A mAb downstream suite. The mAb downstream viral clearance is accomplished by a combination of orthogonal or complementary approaches including filtration, chromatography, and chemical inactivation.

Viral Safety in Monoclonal Antibody Manufacturing

Continued from page 9

demand for clearance means your virus removal might be very effective in one situation, but inadequate in others."

Using well-characterized cell lines, such as CHO, the preferred expression system for monoclonal antibodies (mAbs), is one way to reduce inherent virus loads, assuming the expression system is appropriate for achieving desired product yield and quality.

The second significant source of virus contamination are media components derived from animal sources. This risk, along with the fear of prion contamination, has been the major impetus for adopting animal-free or chemically defined raw materials. Even then, rodent infestations in plants from which raw materials are sourced can result in completely unanticipated viral contamination. "Genentech and Merrimack both experienced bioreactor contaminations by Minute Virus of Mice (MVM), a virus associated with mice," notes Dr. Beattie. "Such adventitious contaminations can result in viruses entering the downstream purification train."

Standard mAb Purification Platform

The standard mAb purification platform includes protein A capture, where significant viral clearance occurs, followed by one or more chromatography steps. Anion exchange chromatography "polishes" process streams of host cell protein and nucleic acid, and can be an important step for viral clearance. Cation-exchange chromatography, which removes process-related impurities like aggregates and charge variants, provides one to three logs of virus removal, which is modest but not insignificant. Viral clearance numbers are logarithmic, so three logs reduction is equivalent to a thousand-fold reduction in virus levels.

However, "if you determine on the basis of product yield or quality that you don't need that process step, you lose its associated clearance," Dr. Beattie points out. "The desire to trim downstream processing to simplify and enhance their productivity carries the risk of eliminating or modifying unit operations, thereby reducing or losing their capacity to remove viruses."

Critical steps in the downstream process are those dedicated to viral clearance, including low pH and/or detergent treatment to reduce levels of enveloped viruses, and virus filtration, which removes both enveloped and non-enveloped viruses. Most processes rely on these dedicated steps to make major contributions to overall viral clearance targets. However, the impact of unit operations on the properties of the molecule can be quite complex. Low pH hold is highly effective for inactivating viruses but is hard on therapeutic proteins and may affect yield, highlighting the interplay of product-quality assessments with requirements for viral clearance throughout downstream purification.

Similarly, upstream-processing conditions will have a direct effect on performance of the downstream unit operations, and higher cell densities and volumetric productivity will also likely affect the amount of virus entering the purification train. These changes can all impact the efficiency of the purification operations for both impurity removal and virus reduction.

A scientist developing a chromatography step at bench-top scale.

Clearance Strategy

Establishing and conducting viral clearance testing for biopharmaceutical customers is the focus of Kathryn Remington, Ph.D., principal scientist focusing on the BioReliance® portfolio of MilliporeSigma. At a previous job

at a large biopharmaceutical company, Dr. Remington collaborated with in-house process-development groups to build viral safety into processes from an early stage, and then evaluated the viral clearance potential of the manufacturing process. Today, services related to viral clearance are largely outsourced, as the time and cost of a dedicated viral safety group and laboratories are beyond the resources of most companies.

A process-centered view of viral safety makes sense since downstream unit operations serve as a "safety net" to clear any adventitious virus that might escape upstream testing. But Dr. Remington cautions that "while some downstream steps provide very good clearance, some don't provide any at all. The level of clearance is process-specific, molecule-specific, and even virus-specific."

Some measures, like chemical inactivation through detergents, inactivate broad classes of viruses, as for example, lipid-enveloped viruses. Low-pH inactivation provides good inactivation of enveloped viruses. But, as mentioned earlier, not all products are stable under acidic conditions. As the pH increases above pH 3.5, inactivation of enveloped viruses becomes less robust. Chromatography clears viruses based on their interaction with the resin. "Each virus has its own isoelectric point and other physical characteristics that make its interaction with resins unique," Dr. Remington says. Removal of virus by filtration is based on size, and high levels of both enveloped and non-enveloped virus can generally be expected to be removed.

Ideally, process developers build in sufficient steps to remove or inactivate as many potential virus threats as possible. "The overall strategy should aim broadly because we don't know *a priori* what viral contaminants we may encounter," says Dr. Remington.

The same step may not always provide the same level of clearance from process to process. "People believe that if a column works great at a certain pH for one molecule that it will provide the same level of clearance in other instances. But that doesn't always work out," she continues. "Sometimes the conductivity or the virus' isoelectric point is not right."

Given appropriate resources, optimizing purification, recovery, and viral clearance is possible. However, development groups typically focus on the first two objectives, then work clearance in afterwards by adding or enhancing certain steps.

"It helps to have sufficient resources to conduct feasibility or even a design of experiment study, to understand the viral clearance potential within certain ranges of operating parameters," Dr. Remington adds. "This will provide greater confidence in implementing future process steps, especially if a platform approach is involved."

Process Intensification

Viral safety often depends on dedicated inactivation and removal steps, and in good part on downstream-chromatography operations with inherent viral clearing capabilities. As biomanufacturers squeeze processes for even greater productivity they must examine if those process improvements affect virus clearance of the individual unit operations.

Michael Phillips, Ph.D., director of next-generation processing R&D, MilliporeSigma, notes that three levels of process intensification could affect viral safety. These are particularly salient for CHO-based mAb manufacturing, where many new ideas in bioprocessing are first implemented.

The first level involves mitigating ➜

An operator setting up a virus filtration step. Virus filtration removes both enveloped and non-enveloped viruses.

Viral Safety in Monoclonal Antibody Manufacturing

Continued from page 11

bottlenecks in fixed facilities, leaving unit operations in place but scheduling or locating them more effectively. The flip side of level one is adopting new technologies to enable faster processing. "You're not really eliminating any step or operation, so the potential impact on viral safety is minimal," says Dr. Phillips.

Compressing processes by connecting unit operations, the second level, presents modest challenges regarding viral safety. However, such strategies, he notes, "need not be serious, provided one remains vigilant that virus removal is not compromised."

The third level of process intensification—continuous processing—is where issues raised by connecting unit operations appear "in spades," according to Dr. Phillips. "Continuous processing is a huge change, a revolutionary development with profound implications for viral safety."

Continuous Processing is a huge change, a revolutionary development with profound implications for viral safety.

As with some revolutions, however, this one will be slow in coming. Although many companies and suppliers are evaluating continuous processing, Dr. Phillips cautions not to expect the coup to occur overnight. "There are regulatory concerns and technical gaps in the ability to implement continuous processing in the near future," he explains. For example, current-generation sensors to ensure reliable operation of continuous processes are lacking, as are control strategies. Dr. Phillips believes that as the technology improves and regulations coalesce around continuous processing, viral safety within that environment will catch up. "But don't expect it for at least five to ten years."

A more modest implementation of continuous processing exists within the confines of individual unit operations. Perfusion cell culture and variants of simulated moving bed chromatography are two examples. Another is inline low pH virus inactivation. The discovery that low-pH incubation could be significantly shorter than the usual one-hour hold, coupled with the inconvenience of standard two-tank virus inactivation, led Dr. Phillips' group to investigate continuous low-pH treatment of process fluid as it emerged from a protein A chromatography capture column, obviating the need for an intermediate incubation/hold step.

When viewed against the backdrop of an entire process, these individual steps less represent continuous processing than optimized or streamlined batch operations in which feedstock enters, is processed, and then awaits the next step. Under ideal continuous operation, process fluids feed directly and continuously into and through operations, and purified product continuously flows out. Continuous or "next-generation" bioprocessing promises huge advances in productivity, but process developers must be aware of how those advances could affect viral safety. The improvements as one progresses along various levels of process intensification also bear a potential cost.

Herb Lutz, global principal consultant at MilliporeSigma, described recent results where a tangential flow filtration-based protein concentration step was performed before flow-through anion-exchange chromatography polishing. "We reduced the volume of the protein solution by a factor of four to make the chromatography column more efficient, but we were obliged to test how this might affect viral clearance," said Lutz. Using this highly concentrated feed, they demonstrated consistent 5 logs clearance of MVM and XMuLV at high product loadings, confirming that viral clearance was maintained in the smaller footprint process.

"We're running processes in new ways," he says. "The data doesn't yet exist to guide processors on the implications of all aspects of how process changes impact viral clearance.

Conventional viral-clearance testing assumes that purification steps behave uniformly throughout their operation. Aliquots of feed are spiked with model virus, subjected to normal filtration or chromatography, and the concentration of virus before and after the operation are measured and results are reported as a log reduction. Lutz, however, does not believe this accurately represents conditions of continuous processing:

"Standard virus-spiking strategies are inadequate when the feed solution changes because a protein peak is coming through or some other event is occurring," he maintains.

To better assess such operations, Lutz developed a technique termed inline spiking, which enables monitoring of viral clearance under more representative conditions, when the process feed changes due to fluctuating concentrations of proteins and salts.

It turns out that in most cases (e.g., during cation-exchange chromatography), virus retention is fairly constant over a wide range of protein concentrations. Nevertheless, the value of inline spiking is that for a minor investment in time it provides a clear answer. "Regulators like that," Lutz adds.

Laying the Foundation for Viral Safety

Mitigating the Risk of Viral Contamination in Vaccines, Cell, and Gene Therapies

Meghaan M. Ferreira, Ph.D.

Viral contamination can result in lost product batches, equipment sterilization costs, and facility shutdowns, costing biotherapeutic manufacturers millions of dollars. Even more importantly, undetected contamination can compromise patient safety and trust.

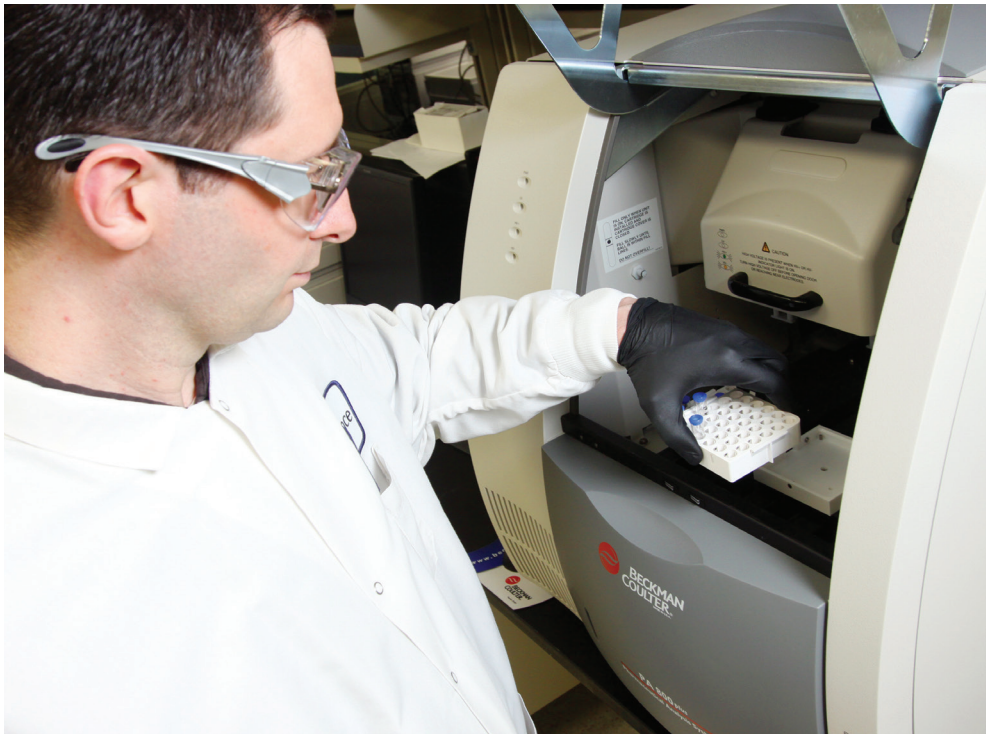
In 2009, the discovery of *Vesivirus* contamination forced a biopharmaceutical manufacturer to shut down a manufacturing facility, which caused a severe shortage of expensive, single-supplier enzyme-replacement therapies used to treat rare genetic diseases. The incident highlighted the importance of having a strong system in place to mitigate the risk of viral contamination, as well as a contingency plan in the unfortunate case that contamination does occur.

In contrast to chemically derived, small-molecule drugs, biologics are generally produced in animal or human cell lines, which carry the risk of virus contamination. While cell culture media and supplements for monoclonal antibody (mAb) production are often chemically defined, the same is not true for vaccines and cell- and gene-therapy products, which generally rely on some animal-derived materials for their production. While a trend toward animal-origin-free materials has begun, it's not always easy or possible to implement, and the use of animal- or human-derived materials significantly increases the risk of adventitious viral contamination.

To reduce this risk, manufacturers rely on three fundamental pillars to support their viral safety strategies: preventing virus entry by careful source-material selection, detection of viral contaminants by multistage testing, and virus removal by multiple operations in downstream purification. This three-tiered strategy provides a solid foundation for viral safety in the production of recombinant protein and mAb therapies, but the one-size-fits-most manufacturing strategies used for protein production often don't work well for non-protein biologics like viral vaccines, cell therapies, and gene therapies delivered via recombinant viruses.

Alternate Approaches for Non-mAb Therapies

The diverse characteristics of these novel therapies make them incompatible with the majority of technologies currently used to inactivate and remove viral contaminants during ➜



Lab scientist testing raw materials to ensure their quality and purity.

Laying the Foundation for Viral Safety

Continued from page 13

downstream purification, thus eliminating one of the three pillars that form the foundation of viral safety in mAb manufacturing. For example, the large size of cell therapies makes nanofiltration and other downstream, size-based separation techniques used to remove virus from protein-based therapies impractical. Gene therapies that use recombinant viruses (also known as viral vectors) to deliver DNA into cells face a similar challenge, since the absence of a sufficient size difference between adventitious and therapeutic virus may also prohibit the use of size-based filtration technologies. Downstream methods traditionally used to inactivate viruses, such as low-pH incubation and gamma- or UV-irradiation treatment, also leave manufacturers without many viable options. Sensitive cell therapies cannot withstand these harsh conditions, and anything that would disrupt a contaminating virus would also likely inactivate vaccines or gene therapies whose effectiveness depends on virus activity.

Although the underlying cause of viral contamination is not identified in the clear majority of occurrences, a few reports have suspected raw materials, such as serum, medium, and trypsin. Since manufacturers often cannot apply virus inactivation and removal systems to the downstream purification of therapies, they are moving to implement treatments for source materials before they enter the manufacturing train. Treatment of media with high temperature short time (HTST) or irradiation with gamma or UV can help reduce virus levels in some components, but not all supplements are compatible with these treatment methods and the equipment can be expensive to install and validate. Newer developments include specially designed filters for quickly and easily processing cell culture media and upstream supplements as well as increased focus on single-use technologies to minimize the likelihood of virus entry from operators or the environment.

A HTST mid-scale unit operation

Novel Filters and Chromatography Media

Many of the downstream virus, clearance technologies simply weren't designed for upstream use, making their performance in upstream applications less than optimal. Viral clearance filters, for example, "Are mostly designed for downstream purification of protein-based therapeutics," commented Priyabrata Pattnaik, Ph.D., head of biologics operations, Asia Pacific, MilliporeSigma. Dr. Pattnaik also mentioned that the R&D team at MilliporeSigma has developed a novel filter specifically designed to handle complex cell culture media.

"[The team] optimized the membrane chemistry and design configuration to adapt the product to deal with media filtration where it can offer high-throughput volumes so that it's economically feasible to implement," he explained.

Merck KGaA, Darmstadt, Germany has also partnered with the DiViNe consortium to develop an affinity chromatography resin for downstream vaccine purification. The DiViNe project, which received funding from the European Union's Horizon 2020 research and innovation program, aims to improve yields, decrease costs, and reduce the environmental impact of vaccine production by employing an affinity-based strategy, similar to the use of Protein A in mAb production. The chromatography resin uses Nanofitin® ligands, with tailored affinities, to capture and elute viral- vaccine products. Affinity chromatography can provide an option for selective removal of adventitious viral agents from vaccine processes.

While current ion-exchange chromatography methods typically work for viral-vaccine and viral-vector purification, Dr. Pattnaik contended that they generally exhibit low capacity and poor selectivity.

Tailoring virus-reduction technolo-

gies to better fit the needs of both upstream and downstream processes of vaccine, and cell- and gene-therapy production exemplifies some of the ways in which manufacturers have adapted their processes to ensure viral safety in these novel classes of biologics.

Viral Safety Pillars

While individual cell and viral therapeutics may be amenable to some virus inactivation and removal methods, "By and large you've lost one of your [viral safety] pillars," remarked Martha Rook, Ph.D., head of gene editing, novel modalities, MilliporeSigma. "There's a much bigger focus on the selection of your source material, on the testing of the product in various stages of manufacturing, as well as, now, the processing conditions themselves."

In addition to placing a greater emphasis on qualifying raw materials and their suppliers, the movement to adopt closed, single-use systems throughout the manufacturing train, from 2,000 L bioreactors to prepacked chromatography columns, has gained traction. Ensuring a closed, aseptic manufacturing process that does not introduce adventitious viruses is important, because "you're not going to have an opportunity to clear them later," noted Dr. Rook.

Biopharmaceutical developers have also started moving toward chemically defined cell culture media for cell-therapy production. Chemically defined media often contain recombinant proteins, cytokines, and/or growth hormones in lieu of animal-derived components, which can cause significant batch-to-batch variation and increased risk of viral contamination.

While chemically defined media mitigates safety risks and generates more robust, reliable processes, its adoption may impact the clinical efficacy of a cell-therapy product, where media and supplements can significantly influence cell phenotype. Finding the perfect recipe for chemically defined media that maintains phenotype, potency, and efficacy of the therapeutic product still relies heavily on scientific →

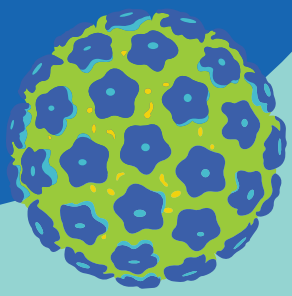
While biopharmaceutical companies work toward establishing better methods to ensure viral safety, they are also ushering in a new wave of novel therapies for diseases with an unmet need.

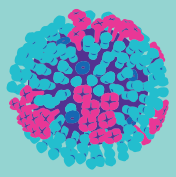
Operator installing a single-use bag in a Mobius® 2000 L single-use bioreactor.

Mobius® FlexReady solution with smart Flexware® assemblies for chromatography and prepacked Chromabolt® chromatography column.

prevent, detect, remove

Current and Future Solutions for Biopharmaceutical Virus Safety



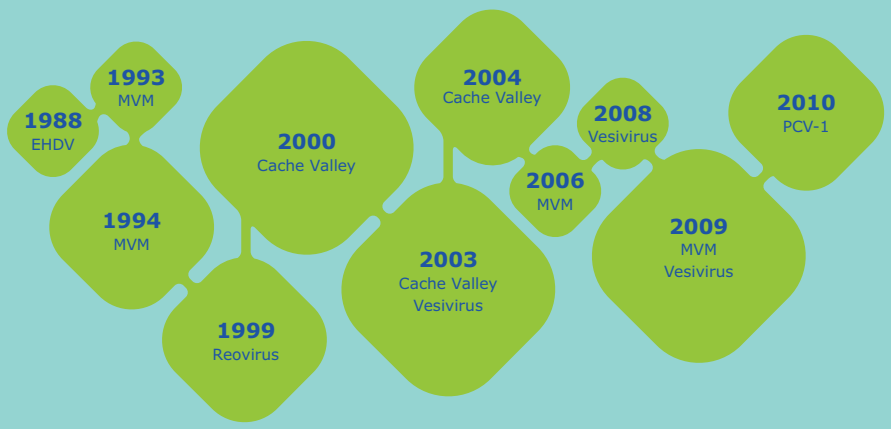


MilliporeSigma

A virus contamination can shut down a biopharmaceutical plant for months impacting manufacturing operations, causing significant business disruption and ultimately threatening drug supply. Fortunately, a range of technologies are available today to help prevent virus contamination and assure an efficient and safe biopharmaceutical production process.

1 Adventitious viruses

A Constant Process Threat



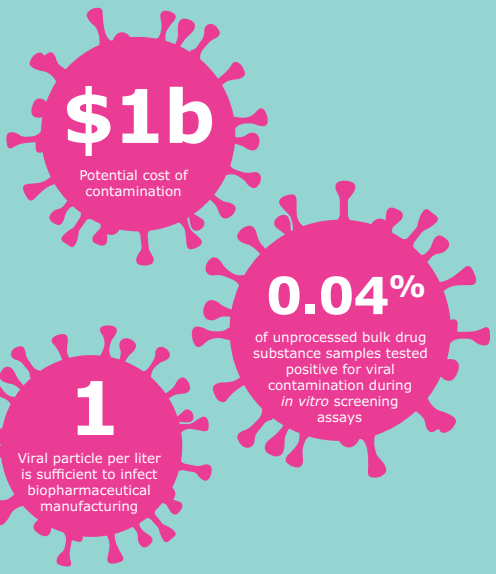
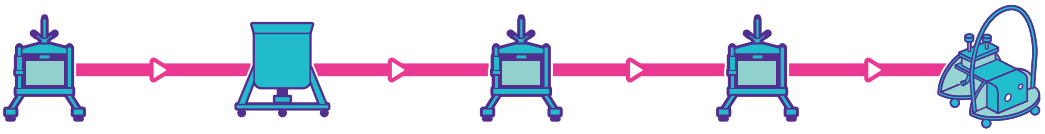


Fig.1 Reported major viral contamination events in biopharmaceutical manufacturing

2 Traditional solutions

Virus safety solutions that remove virus from monoclonal antibody and recombinant protein production are well understood.



	Affinity Chromatography	Low pH	Cation Exchange Chromatography	Anion Exchange Chromatography	Virus Filtration
Parvovirus LRV	~2-3	n/a	~1	~4-5	~4-5
Retrovirus LRV	~4-5	4-6	~2-3	~4-5	~5

Fig.2 Expected viral clearance by manufacturing unit operation, log reduction value (LRV).
Miesegaes G., Lute, S., Brorson K., (2010) Analysis of Viral Clearance Unit Operations for Monoclonal Antibodies. *Biotechnology and Bioengineering* Vol 106, No 2, June 1 2010 p 238-246

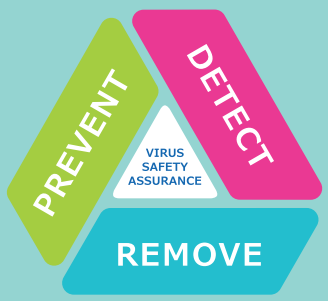
Downstream chromatographic purification

Downstream processing separates the protein of interest from cell culture harvest and results in a purified, concentrated molecule with low levels of impurities. Various technologies with a variety of base media, ligands and formats offer multiple options for purification. Although purification is the primary goal, reliable virus removal is also required to meet the virus safety needs of the downstream process.

Downstream virus filtration

Robust viral clearance can be maintained during virus filtration following planned or unplanned process interruptions, assuring performance and consistency of this critical virus reduction operation. Flexible prefiltration options enable superior mass capacity across a broad range of molecules and conditions to meet the needs of today's biomanufacturers.

3 What's next to Minimize risks?



A risk mitigation strategy that includes the prevention, detection, and removal of virus contamination will help ensure virus safety.

Fig.3 Virus safety assurance

	Expression System	Raw Materials	Media Preparation	Cell Culture	Purification
Prevent	• Genetic modification of host cell	• Chemically defined raw materials • Treatment of high-risk components	• Virus barrier filtration • High Temperature, Short Time (HTST) Treatment		
Detect		• Adventitious virus testing • Next Generation Sequencing (NGS)	• PCR testing • In vitro testing • Next Generation Sequencing (NGS)		
Remove			• Low pH/chemical inactivation • Chromatography • Virus filtration		

Fig.4 Various technologies help minimize virus contamination risks throughout the process

A diverse range of new technologies further minimize the risk of introducing virus contamination into biopharmaceutical production, including virus-resistant engineered CHO cell lines, novel filters designed specifically for cell culture media, and innovative technologies for sensitive detection of unknown viruses. More traditional virus filtration technologies have been augmented by new prefiltration options enabling more efficient processing of a broader range of feed streams.

Prevent

Gene Editing Technology created engineered CHO cell lines that are resistant to MVM, one of the main virus contaminants in the industry.

New barrier technologies like **filtration products designed for cell culture media** offer easy to use and implement solutions for risk reduction.

Detect

Next Generation Sequencing opens doors for more rapid, sensitive detection of virus contaminants

Remove

Prefilters remove plugging species from mAb processes significantly improving filtration capacity of the device while maintaining high parvovirus removal and flux.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

Laying the Foundation for Viral Safety

Continued from page 15

discovery and, as a result, remains a long-term strategy for most companies.

Screening the product for adventitious viruses throughout the manufacturing process forms the third pillar that safeguards against viral contamination. Cell therapies in particular may require testing methods with high enough sensitivity to detect viral contamination, despite the smaller volume of material available for testing compared to more traditional therapeutics.

"Classic viral tests are still [used]—they're still defined, they're still approved, they're still relevant," affirmed Alison Armstrong, Ph.D., senior director, global head of field development services focusing on the BioReliance® portfolio of MilliporeSigma. "It's really about where modifications may be applied…to address whether a virus, and perhaps a novel virus, is present."

Virus Detection Assays

The amount of knowledge needed a priori to detect adventitious viruses depends on the different techniques employed. PCR-based methods require a high degree of homology (usually 95%) to the sequence selected for testing and may not detect a novel virus, even if it comes from the same family as the virus of interest.

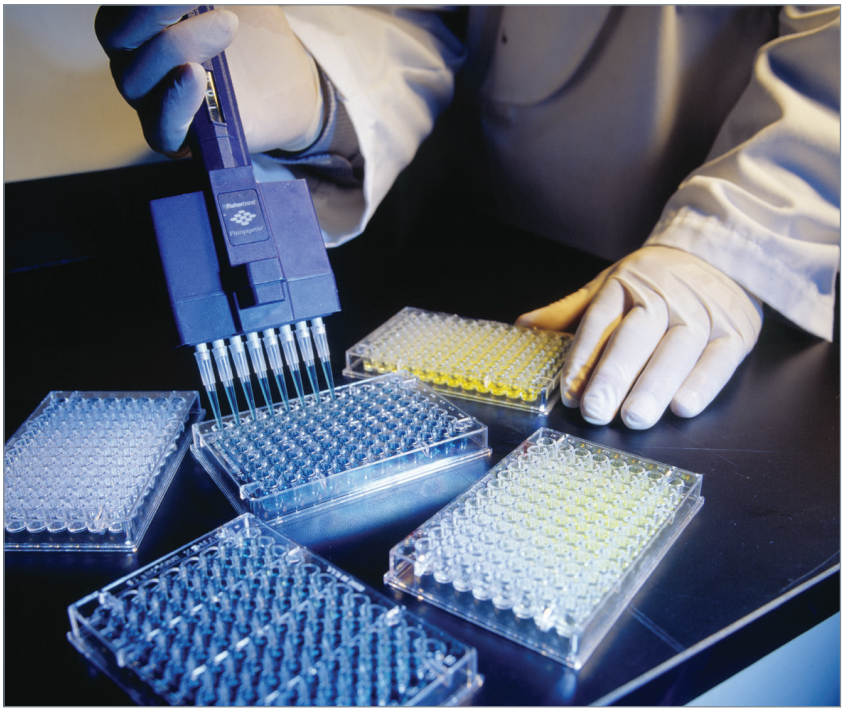
Culture-based infectivity assays offer a broader range of detection for nonspecific viral agents. These assays expose indicator cell lines to a test sample, which then undergoes an observation period to determine if an infection event has occurred. However, infectivity assays are not completely agnostic as cell-line dependent variations in susceptibility to a particular pathogen can still bias detection.

Testing for adventitious virus with cell-based assays in viral vaccine products can also be challenging because the viral vaccine must be completely neutralized before testing. "[It's] a really hard assay to do," noted Julie Murrell, Ph.D., head of cell therapy bioprocessing, MilliporeSigma. "It takes a while to do it, [and] it takes a skilled operator to make the call on whether or not a cell is infected" with adventitious virus.

In contrast, Next Generation Sequencing (NGS) offers a nondirected, completely agnostic approach that makes it a powerful tool for virus detection. It's taken a while for the technology to make a home for itself in the industry. Both the high computational power and the extensive bioinformatics required to transform sequencing data into useful information remain drawbacks of the technology. But, unlike directed methods, NGS can detect novel viruses without a priori knowledge. "The universe of viruses is large, and new viruses are constantly being identified or discovered," said Dr. Murrell, "so what we're testing for today might be different than what we're testing for in the future."

The discovery and development of biologics offers hope to many patients, but only with security of supply. The shortage of medication caused by the Vesivirus contamination in 2009 highlighted the criticality of viral safety to the industry and patients alike. While biopharmaceutical companies work toward establishing better methods to ensure viral safety, they are also ushering in a new wave of novel therapies for diseases with an unmet need, like rare genetic conditions and advanced cancers. Even as companies work to address the viral-safety challenges these therapeutics bring with them, "The risk-benefit ratio is still very much in the balance of benefit to the patient," Dr. Rook reminded us.

As these new modalities advance and expand to include a broader spectrum of indications, Dr. Rook is confident that manufacturers will mature and grow with them to ensure a safe product: "[Viral-safety strategies are] going to evolve. We're not going to stand still. We'll see these kinds of advances, and they'll be needed as the use of these types of therapies expands."



Scientist involved in performing cell line characterization testing.

Keeping Up with Viral Safety Trends in Bioprocessing

Next-Generation Sequencing (NGS) and Quality by Design (QbD)

Kristen Slawinski, Ph.D.

Viral safety is a key component to assuring the safety of biopharmaceuticals for human use. Manufacturers need to verify that their products are free of harmful viruses, while regulatory agencies are tasked with upholding standards for viral safety for all types of biopharmaceutical products. As live cells are used to express the biopharmaceutical product, and many of the components used in cell growth are of animal origin, the risks of virus contamination are high. New technologies and methodologies are emerging for both producing and testing biopharmaceuticals, so the industry is entering a new era of viral safety assurance.

The High Cost and Likely Suspects of Viral Contamination

"If virus is detected anywhere in a biomanufacturing process, the entire process must be shut down and an intensive investigation embarked upon," says Bala Raghunath, Ph.D., director of global manufacturing sciences and technology at MilliporeSigma. A contamination incident in 2009 triggered a facility decontamination, extensive investigations of root cause, and penalties from concerned health authorities, not to mention the significant loss in revenues as a result of the plant shutdown. "This impacted supply of critical drugs which, in turn, affected patient access," says Dr. Raghunath. Several reports detailed the incidence of this contamination.

Raw materials have often been implicated in viral contamination incidents and there is a general acceptance of their vulnerability to virus contamination. Testing cell culture media for the presence of virus is inherently constrained by assay sensitivity and an inability to detect low levels of virus contaminants. As a consequence, manufacturers are considering adding steps to inactivate or remove potential virus contaminants from cell culture medium and other raw materials used in upstream manufacturing processes.

"Some companies have evaluated high temperature short time (HTST) ➜

Lab scientist testing raw materials.

Keeping Up with Viral Safety Trends in Bioprocessing

Continued from page 19

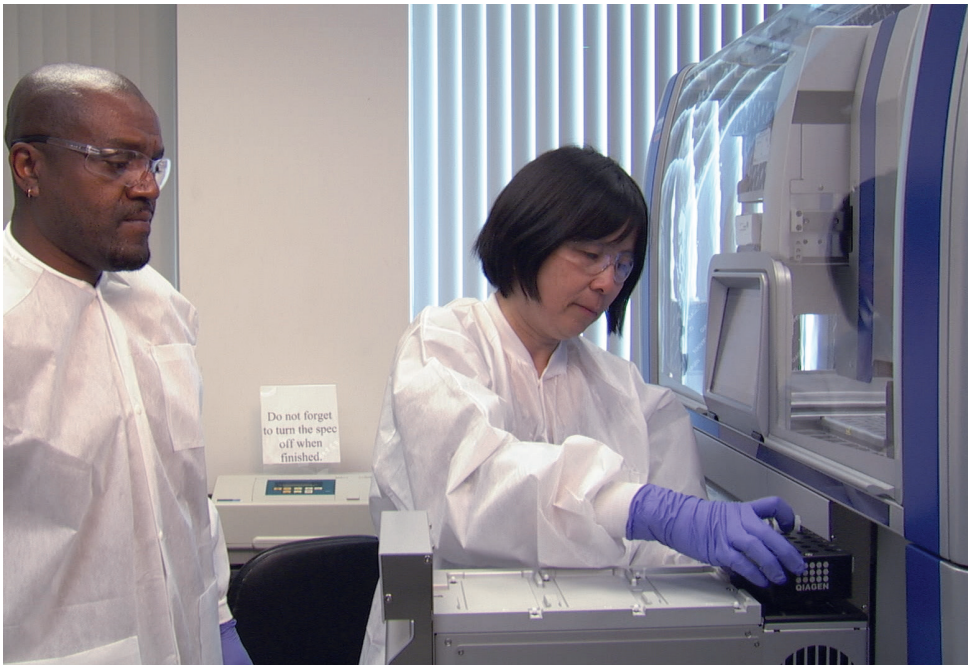
pasteurization methods to kill pathogens in cell culture media," says Dr. Raghunath. "However, not all media components are stable at high temperatures. Further, HTST requires some significant initial investment and equipment, so only a few companies have taken that approach. More recently, companies have developed novel filters designed for cell culture-media treatment. Cell culture media is at risk for introducing viral contamination into a bioreactor, so having that viral filtration step is considered a good way to enhance viral safety assurance in the manufacturing process."

In addition to cell culture media, cell banks used for manufacturing biotherapeutics are also considered high risk for introducing contamination and need to be well characterized before starting manufacturing.

"There is extensive characterization of the master, working, and end of production cell banks before bioprocessing," said Kathryn Remington, Ph.D., principal scientist focusing on the BioReliance® portfolio of MilliporeSigma. "Companies need to verify that the cells are the type that they think they are and also look for purity of the cells. They'll then need to be screened for bacteria, fungi, mycoplasma, and viruses."

Martin Wisher Ph.D., global head of regulatory affairs focusing on the BioReliance® portfolio of MilliporeSigma added that "*in vivo* studies need to be completed in the master cell bank and end of production cell line. You want to be sure that your cell banks are free of viral contamination. Testing the bulk harvest with *in vitro* assays gives you further evidence that the cell banks were clear and gives you assurance that there is no other contamination coming in through the process."

Regulatory testing standards for cell banks and bulk harvest are outlined in the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH Q5A) guidelines. The ICH brings together the regulatory authorities from Europe, Japan, and the U.S. as well as industry experts with a purpose of providing harmonized recommendations and technical guidelines for product registration. Current guidelines recommend extensive *in vivo* testing for viral safety purposes in bioprocessing. But, with the development of new technologies, the industry is questioning the utility and relevance of these more traditional tests.



Scientists involved in performing cell line characterization testing.

In with NGS, out with *In Vivo* Analyses

"One technique that the industry is hoping to get rid of is the *in vivo* assay," says Dr. Wisher. "In the last few months, there has been a move to rewrite specific ICH guidelines to remove the requirement for *in vivo* testing. The industry wants to reduce the number of animals used in pharmaceutical testing in general. The old, classical *in vivo* techniques made sense when there wasn't an option to use tissue culture, but they're not as sensitive as people thought they were."

An emerging technique that could help to replace at least some *in vivo* testing is next-generation sequencing (NGS), otherwise known as massively parallel sequencing or "deep" sequencing. NGS is a technique that allows unbiased sequencing of all DNA or RNA in a given sample, and has been commonly used in a research setting for over a decade, but is only more recently being implemented for viral safety testing.

"Traditional testing will only test for a panel of viruses that we know can impact human health," explained Colette Coté, Ph.D., principal scientist focusing on the BioReliance® portfolio of MilliporeSigma. "But the reality is that there may be emerging viruses that we haven't identified that can also affect human health, Zika being a perfect example. NGS enables a deeper search to look for viruses that we may not have known about."

As promising as the technique is for giving more insight into potential viral contaminants, regulatory guidelines are slow to adopt.

"The industry is trying to get their hands and minds around the technology," continued Dr. Coté. "Consistency, robustness, reproducibility and sensitivity need to be shown. They need answers to questions about what the technology can do, what the limitations and advantages are, and how it compares against current technologies." When these questions are answered, regulatory expectations and guidance will likely change.

The use of NGS for testing biologics poses greater challenges as compared to standard PCR or other molecular tests due to its technical complexities and requirement for Big Data bioinformatics. To address these concerns, the Advanced Virus Detection Technologies Interest Group (AVDTIG) was formed as a joint effort by regulatory and industry scientists to share data and experiences using advanced virus-detection technologies such as NGS. "There are about 30 companies working in the AVDTIG right now," said Dr. Wisher. "This year, the group is publishing papers that will discuss best practices for NGS methods and data analysis. They're also developing a curated database of useful sequences for viral safety studies as well as making a number of standard purified virus preparations so that spiking studies can be done to get a better idea of the sensitivity of the technique."

Although regulatory agencies will not yet accept NGS as a replacement for standard virus detection tests, the technology is already impacting industry expectations and standards. "Last month at a viral safety meeting, Sanofi announced during a presentation that they will be using NGS for screening cell lines and vaccines and submitting that data along with all of the conventional testing that was done for all new products," said Dr. Wisher.

Viral Safety Testing in Quality by Design

Another trend in the industry, which has been introduced and consistently encouraged by regulatory authorities is the implementation of Quality by Design (QbD) approach in process operations. The QbD approach encourages an understanding of how raw materials and manufacturing processes impact the critical quality attributes of the product, i.e., attributes that impact the safety, efficacy, and quality of a drug product. Ultimately, a design space is defined, which represents the operating range, within which the process meets the critical quality attributes of the product. The "absence of virus" can be considered a critical quality attribute that impacts the safety of the drug product.

"The QbD approach enhances a manufacturer's understanding of their process and unit operations as well as the impact of operating parameters on quality attributes," explained Dr. Raghunath. "Following QbD methodology, we have put together a knowledge base that details the impact of various process conditions and operating parameters on the viral clearance performance of a downstream virus filter. The impact of parameters that can typically change during the process, such as pressure, conductivity, pH, protein concentration, and membrane lots, on virus retention is reported. Understanding the effects of these parameters on quality attributes like virus retention allows those parameters to be controlled within a given range."

Using the principles of QbD for viral safety testing results in a more thorough understanding of the design space for viral clearance unit operations and the appropriate controls that are needed to maintain the operating parameters. Key to the methodology is upfront identification of risk factors and incorporation of testing protocols to manage those risks, and NGS helps to identify viral contamination risk. "When using novel substrates in bioprocessing, extensive NGS characterization can be performed on several lots of the raw materials to identify potential contaminants that may be present," said Dr. Remington. "This can help focus testing so that manufacturers have a rational testing strategy for specific contaminants, and appropriate assays can be designed."

Integrating sensitive new technologies for viral detection into a QbD approach to biomanufacturing provides confidence for both the manufacturer and regulatory agency that the risks of virus entry to the process are minimized, low levels of virus can be detected, and the process parameters are controlled to assure the expected levels of virus removal in the downstream process, thereby assuring the integrity and safety of the drug product. ■

As promising as the technique is for giving more insight into potential viral contaminants, regulatory guidelines are slow to adopt.

Upstream Virus Safety: Protect Your Bioreactor with Media Filtration

Christina Carbrello Ph.D., David Nhiem, Mary Priest, Kimberly Mann, and Trish Greenhalgh Ph.D.

MilliporeSigma

Biopharmaceutical manufacturing processes involve a multilayered approach to microbial and virus testing to assure that the drug product is safe for human use. Screening raw materials, testing in-process intermediates, and demonstrating the virus removal capabilities of the downstream process are critical to biosafety assurance.

However, despite careful screening of raw materials, there remains a risk of introducing adventitious agents into bioreactors, which could impact manufacturing operations, cause significant business disruption, and ultimately threaten drug supply to patients.

Various technologies may be used to minimize this risk. One of these, filtration, is a point-of-use operation that is easy to implement in the upstream process. This tutorial summarizes the performance of a filter specifically developed for virus removal from chemically defined cell culture media. The Viresolve® Barrier Filter removes high levels of virus, mycoplasma, and bacteria without impacting cell growth, antibody titer, or protein quality. The filter has robust performance over a broad range of conditions and offers an effective, easy-to-implement solution for media treatment.

Risk reduction for viral contamination of upstream processes has traditionally relied on careful sourcing of raw materials, screening cell banks for adventitious virus, and control of facilities and workflow. Despite these precautions, bioreactor contaminations have occurred, resulting in significant disruption and cost for the companies involved. More recently, other options for virus reduction in upstream applications have been employed, but generally require costly investment and are often not suitable for all media components.

Filters specifically designed for upstream processing offer an alternative to capital-intensive methods, using proven membrane technology to assure robust, broadly effective, size-based virus removal *(Table)*.

The Viresolve® Barrier Filter can be integrated into single use or stainless steel processes, and can be used in place of a 0.1 µm or 0.2 µm sterilizing-grade filter; high retention (above the detection limit) has been demonstrated for large virus, mycoplasma, and bacteria *(Figure 1)*.

The graph *(Figure 2)* illustrates sustained high level of retention for minute virus of mice (MVM), a relevant small virus contaminant, during extended processing times for two different membranes. Membrane made near the limit of the manufacturing window shows MVM retention of approximately four logs sustained over 8 hours of processing. Typical nominal membrane shows over five logs of MVM retention over the same time period.

Virus retention across the Viresolve® Barrier Filter was evaluated at a range of processing conditions with different representative cell culture media. The filter is designed to retain a minimum of four logs Phi-X174, which is used as a surrogate organism for MVM. Virus retention remains high across a range of operating pressures and pH levels, even after extended processing times *(Figure 3)*.

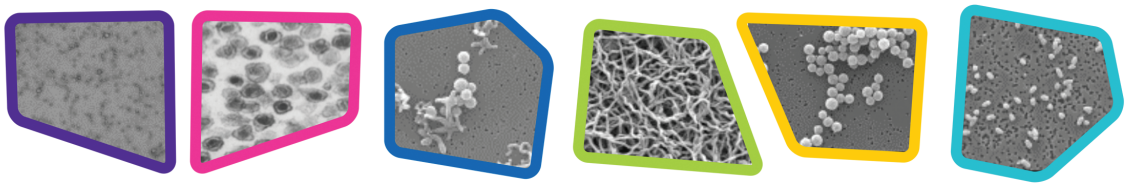
Virus filters designed for downstream applications are inefficient for processing cell culture media. *(Figure 4A)*. The Viresolve® Barrier Filter leverages the proven technology of the Viresolve® platform with asymmetric polyethersulfone (PES) membrane ➜

Comparison of Upstream Virus Prevention Technologies

Technology	Pros	Cons
High Temperature Short Time (HTST)	Robust clearance Point-of-use Cost-effective at large scale	Conflicting clearance data Media compatibility Not cost-effective at small/mid scale
UV-C (254 nm)	Point-of-use	Virus dependent clearance Media compatibility Challenging at large scale
Irradiation (25-40 kGy)	Cost-effective	Virus dependent clearance Media compatibility Not point-of-use Best with small batches
Virus filtration with optimized upstream filters	Robust, sized based clearance Familiar format Compatible with most media Point-of-use	Not effective if media contains unusually large critical species

Table.

Retention of a Broad Panel of Microorganisms with Viresolve® Barrier Filter



Minute Virus of Mice
Relevant contaminant
Target organism small virus
Typical LRV above 4
Worst case LRV ≥ 3

Murine leukemia virus
Model large virus
LRV >6.1

M. orale
Relevant contaminant
Can penetrate 0.1 µm filters
LRV >8

L. illini
Model spirochete bacteria
Can penetrate 0.1 µm filters
LRV >8

A. laidlawii
Standard mycoplasma model organism
Model organism for 0.1 µm filters
LRV >8

B. diminuta
Standard model bacteria
ASTM® F838-05 test organism
LRV >8

Figure 1.

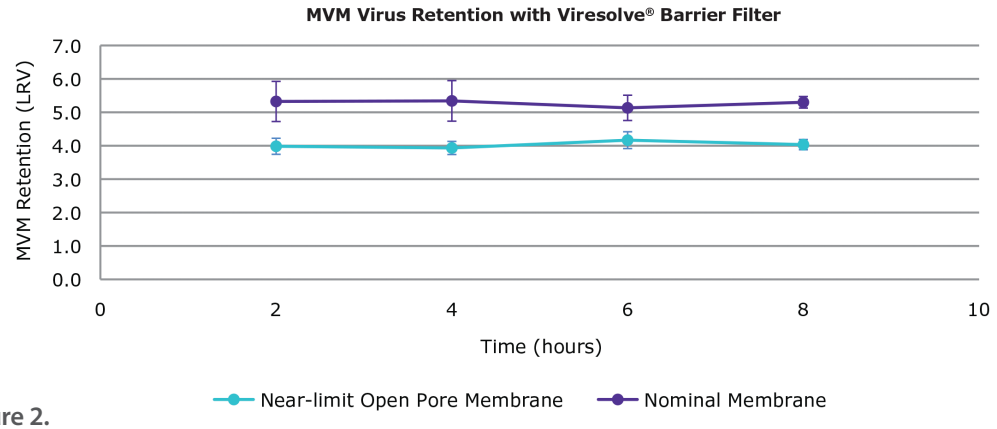


Figure 2.

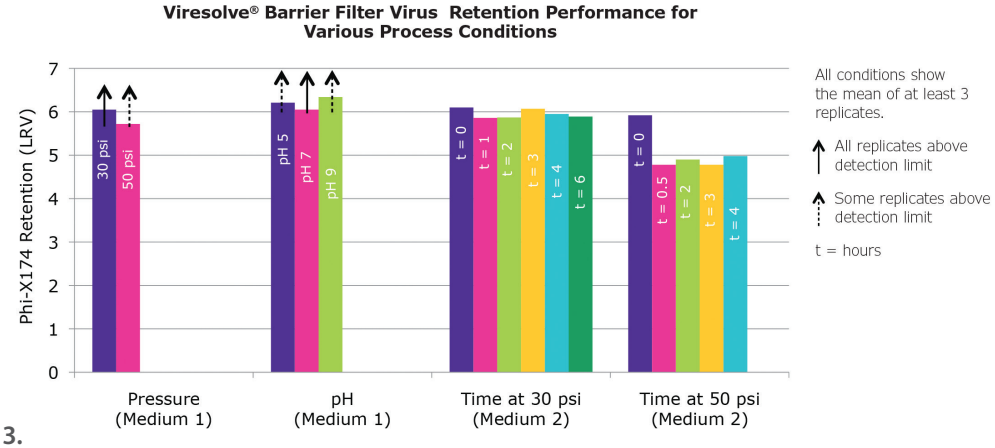


Figure 3.

Upstream Virus Safety: Protect Your Bioreactor by Media Filtration

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technology and a novel secondary chemistry formulated for optimal processing of chemically defined media. This unique filter provides good volumetric throughput across a range of "off the shelf" and proprietary media *(Figure 4B)*.

Comprehensive analysis (mass spec or amino acid and soluble vitamin HPLC, NMR, and ICP-OES) of two cell culture media and their respective feeds before and after filtration through Viresolve® Barrier Filter indicated no changes in media composition that could be attributed to filtration with the Viresolve® Barrier Filter.

Cell culture was performed using filtered media in shake flasks (Cellvento™ CHO-200 medium with MAb01) or Mobius® 3L Bioreactors (Ex-Cell® Advanced™ CHO medium with MAb02). No significant changes in viable cell densities *(Figure 5A)* or antibody titers *(Figure 5B)* were observed, and analysis of antibody charge heterogeneity, aggregate profile, and glycan profile indicated no changes as a result of the cell culture media filtration (not shown).

Summary

Risk-based analysis of bioprocess manufacturing processes highlights weaknesses in design and, therefore, offers opportunities for improving specific elements that can impact virus safety. The Viresolve® Barrier Filter is specifically designed to reduce risk early in production by adding a final layer of protection before the bioreactor, enhancing existing materials sourcing, selection and facility control processes. The filter is easy to use, does not impact cell culture processes, and provides a high level of virus removal across a range of conditions, increasing confidence that microorganisms will not be introduced to the bioreactor. ■

Christina Carbrello, Ph.D., is a senior scientist; David Nhiem is a development engineer; Mary Priest is a lab manager; Kimberly Mann is a senior scientist; and Trish Greenhalgh, Ph.D., is an R&D manager, MilliporeSigma.

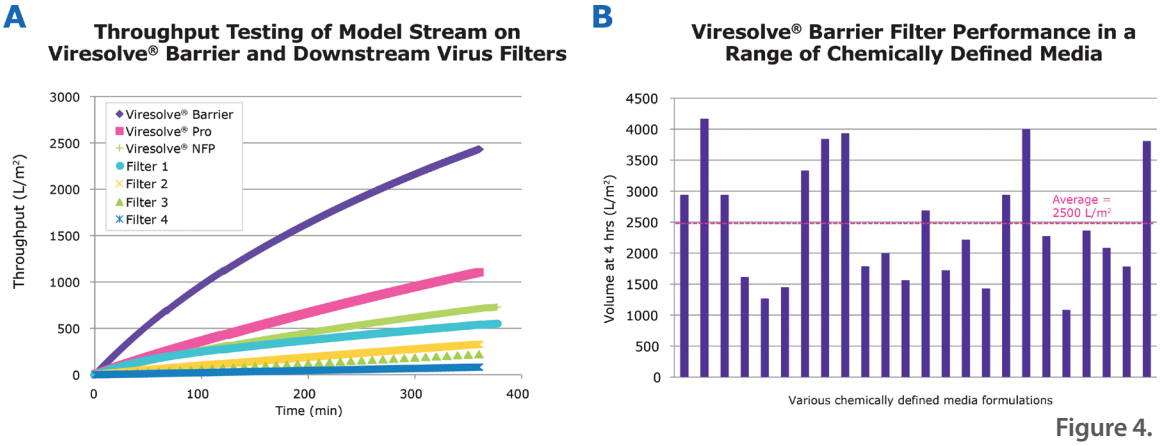
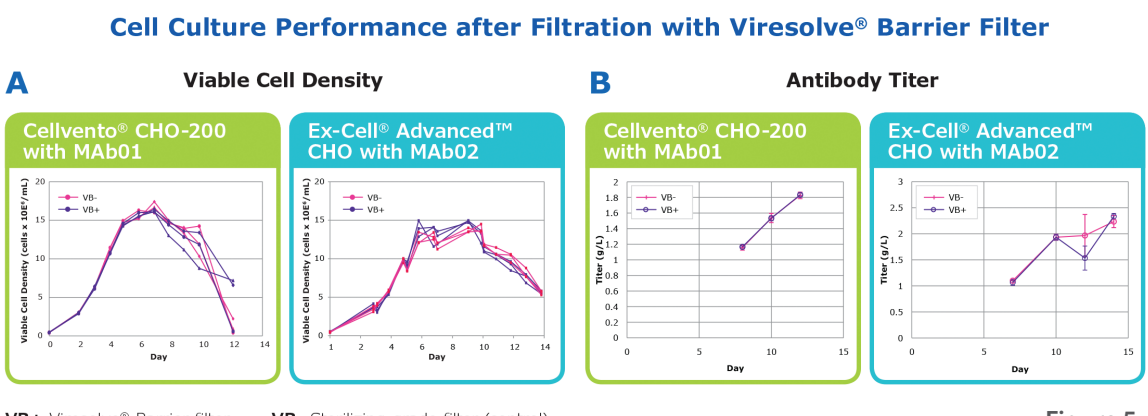


Figure 4.



VB+ Viresolve® Barrier filter **VB-** Sterilizing-grade filter (control)

Figure 5.

Utility of GMP Next-Generation Sequencing (NGS) for Biosafety Assessment of Biological Products

Colette Côté Ph.D., Lakshmi Viswanathan, Sindy John, and Audrey Chang, Ph.D.

The advent of next-generation sequencing (NGS), also referred to as massively parallel or deep sequencing, affords a radically different approach to the challenge of identifying and characterizing known and unknown agents (sequences) with precision and sensitivity. By delivering significantly more data than traditional Sanger-based sequencing methods, NGS opens a range of possibilities for the analysis of diverse DNA and RNA populations.

NGS does not require any prior knowledge of the sample sequence; the technology is capable of detecting all sequences in a sample, whether known or not. An NGS library is constructed from sample nucleic acid and then sequenced *(Figure 1)*. Comparison of a sequence to target sequences or to libraries of known reference sequences using bioinformatics programs reveals identities. Identification of novel sequences is made possible by virtue of homology to known elements/sequences.

By combining custom sample preparation with tailored sequencing and bioinformatics, NGS is ideal for the characterization of biological products (e.g., viral vaccines/products, raw materials, cell lines used in biomanufacturing, and final drug products [*Figure 2*]) as part of a Quality by Design (QbD) approach.

NGS is also particularly well suited for biosafety testing, including the identification of unknown contaminants in biological samples or systems (e.g., those that result in bioreactor/fermentor failures or unexpected morphological changes/cell death during cell culture). In such instances, a rapid investigation, combined with the ability to detect contaminants without bias or prejudice, is essential and NGS can be the critical first step for contamination remediation.

NGS continues to prove itself, not only as a key supplementary tool, but also a key alternative method to address testing requirements specific for virus-based therapeutic products. Virus-based therapeutic products *(Figure 3)* are viruses that are converted into therapeutic agents by reprogramming them to treat disease. They can be grouped by application:

- Viral vaccines—viruses designed to prevent replication and elicit an immune response
- Oncolytic virotherapy—viruses that selectively target cancer and tumor cells and elicit an antitumor immune response
- Viral gene therapy—viruses that deliver therapeutic genes to cells with genetic malfunctions
- Viral immunotherapy—viruses that introduce specific antigens to a patient's immune system

Plenty of regulatory guidance exists around the manufacture of viral-based medicinal products. FDA and EMEA guidance documents and reflection papers outline testing →

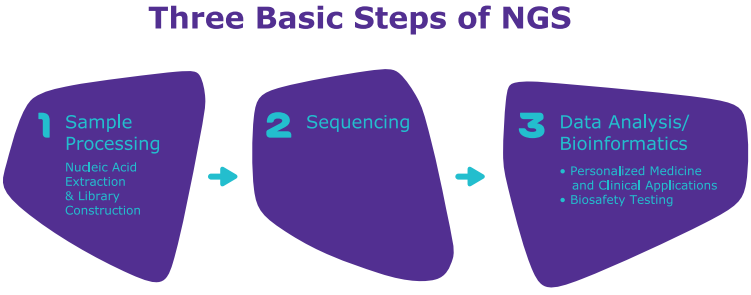


Figure 1.

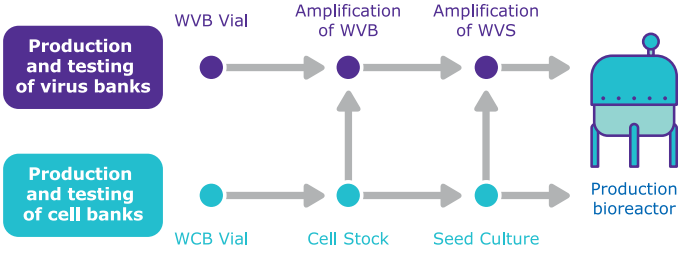


Figure 2.

Utility of GMP Next-Generation Sequencing (NGS) for Biosafety Assessment of Biological Products

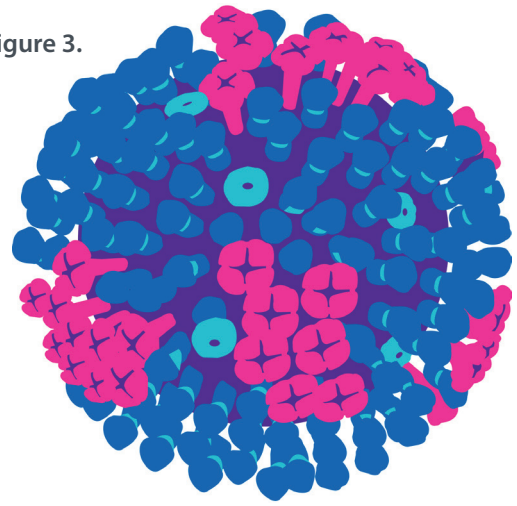
Continued from page 25

strategies and recommendations. While use of NGS is not specifically defined, it may be inferred under use of "state-of-art" technologies.

Challenges for Traditional Testing of Viral Products

Traditional tests for adventitious virus are lengthy (e.g., sterility, 14 days; mycoplasma, 28 days; [*Figure 4*] and RCR, 28 days). Although they may detect a contaminant, they generally do not directly identify it. Often, only small lots with limited sample volumes of viral-based therapeutic products are produced, resulting in limited availability of starting material for process, product, and test-method development. Another testing challenge for these types of products is the lack of reference standards and the requirement for producing neutralizing antisera, required for many traditional assays. NGS offers opportunities for circumventing some of these challenges by offering a consistent and sensitive method compatible with a diverse range of products to test for and identify adventitious virus *(Figure 5)*.

Figure 3.



Specific NGS Applications for Virus Product Safety and Characterization

One key application of NGS is identity testing and variant detection, which is of great value when large or difficult-to-sequence genomes are evaluated, or when structural and rare variants are involved *(Figure 6)*.

Equally important is its application in contaminant detection. Here, NGS allows both detection and identification of viral, bacterial, or fungal sequence signatures, of both known and unknown agents *(Figure 7)*.

In summary, NGS has clearly emerged as an effective molecular tool with a wide range of applications in biosafety testing and biomanufacturing. NGS offers not only a supplementary method, but also a novel alternative testing strategy, enabling solutions where traditional testing approaches struggle or fail. Regulatory guidance driving the use of NGS for specific applications is still a work-in-progress. However, the technology can clearly be developed for, and applied in, a regulatory setting, and meet, if not exceed, the requirements and expectations. ■

Colette Côté, Ph.D., is principal scientist, head of next-generation sequencing and bioinformatics, development services; Lakshmi Viswanathan, serves as senior scientist, next-generation sequencing, development services; Sindhu John is bioinformatics engineer supervisor, next-generation sequencing, development services; and Audrey Chang, Ph.D., serves as head of development services, at the BioReliance® portfolio of MilliporeSigma.

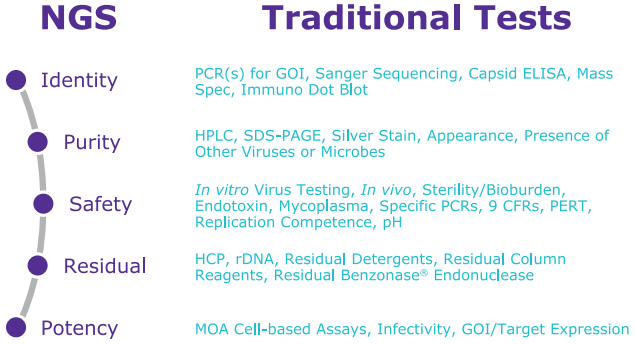


Figure 4.

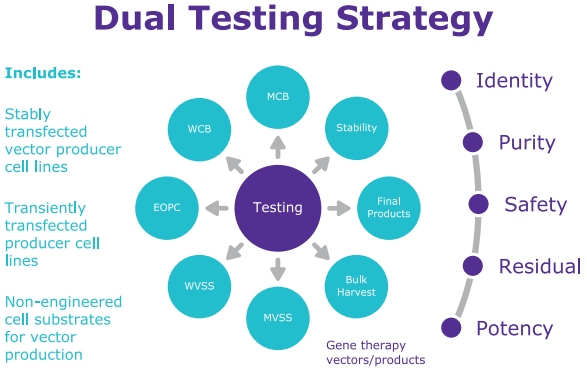


Figure 5.

Figure 6.

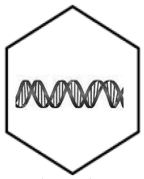
ssAAV

Total # of Reads Used for Mapping	Reference Sequence/ Accession Number Used for Mapping	Accession Length (Bases)	Total # of Mapped Reads	% of Population	Average Depth of Coverage	Consensus Length Generated By Mapping (Passing Quality Bases)	% Reference Coverage	% Similarity to Reference	Total Number of Unmapped or Low Quality Positions
46,785,021	XXX	4,226	46,084,621	98.5	2,993,955	4,221	99.88	100	5*

scAAV

Total # of Reads Used for Mapping	Reference Sequence Used for Mapping	Reference Length (Bases)	Total # of Mapped Reads	% of Read Population Mapped	Consensus Length Generated By Mapping (Bases)	% Reference Coverage	% Similarity (Identity) to Reference	Depth of Coverage (Reads)		
								Ave	Min*	Max
38,031,139	XXX	6,042	36,555,478	96.12	6,042	100.00	99.87	1,490,411	608	6,867,072

Enables sequencing through highly complex secondary structures

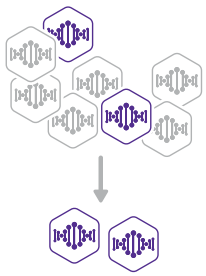


Adenovirus

Total # of Reads Used for Mapping	Reference Length (Bases)	Total # of Mapped Reads	% of Population Mapped	Consensus Length Generated By Mapping	% Reference Coverage	% Consensus Similarity (Identity) to Reference	Average Depth of Coverage Across Consensus	Maximum Depth of Coverage Across Consensus	Minimum Depth of Coverage Across Consensus	# Variants Detected
39,185,260	36,374	39,108,410	99.80	36,374	100.00	100.00	139,892.05	442,287	3,082	0

Enables variant detection at the very ends of linear genomes

Reference	Position	Reference Base	Variant Base	Number of High-Quality Ref (fwd)	Number of High-Quality Ref (rev)	Number of High-Quality Var (fwd)	Number of High-Quality Var (rev)	Variant Freq (%)	Variant Type
AdVAV	3,500	Complex Variant See description in section 5.4 below		2	7	90293	116444	100.0	Insertion
AdVAV	3,502			6	11	12646	603	99.87	Insertion
AdVAV	3,503			17816	2472	89504	117456	91.07	Insertion
AdVAV	3,504			19213	2794	89823	117057	90.39	Insertion
AdVAV	4,943	G	C	15	18	116869	107630	99.99	Substitution
AdVAV	8,774	G	A	45	44	57737	24772	99.89	Substitution
AdVAV	10,582	TAGA	TA	679	2014	2959	828	58.44	Deletion
AdVAV	10,584	GACC	GC	9	5	66010	37060	99.99	Deletion
AdVAV	10,586	C	G	8	8	6	3	36.00	Substitution
AdVAV	11,275	T	C	42	17	128119	58365	99.97	Substitution
AdVAV	17,378	G	C	22	32	72120	100564	99.97	Substitution
AdVAV	18,745	Complex Variant See description in section 5.4 below		2	11	58388	77561	99.99	Deletion
AdVAV	19,474	T	A	205	97	130680	144544	99.89	Substitution
AdVAV	19,504	T	A	23	43	117989	148380	99.98	Substitution
AdVAV	19,648	G	A	23	49	81800	193698	99.97	Substitution
AdVAV	19,649	A	G	19	41	76976	189932	99.98	Substitution



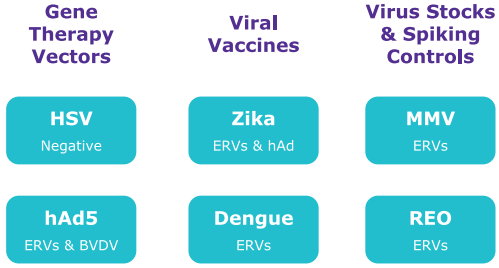


Figure 7.

Prevent, Detect, Remove Virus

Is your bioprocess protected from viral threats? Do you know how safe your raw materials are? Do you have a holistic viral safety strategy to protect your process from start to finish? If not, we're here to help. Our comprehensive offering of viral safety products and services will enable you to develop, implement and validate your process to meet regulatory requirements.

Viral Safety—Prevent

Viresolve® Barrier filter for cell culture media

HTST treated glucose for bioreactor feeds

Centinel Intelligent Virus Defense™ technology (MVM-Resistant CHO cells)

Viral Safety—Remove

Mobius® MIX systems with Emprove® products for low pH and solvent/detergent treatments

ProSep®, Fractogel® and Eshmuno® chromatography resins

Viresolve® Pro Solution

BioReliance® viral clearance services or testing

Viral Safety—Detect

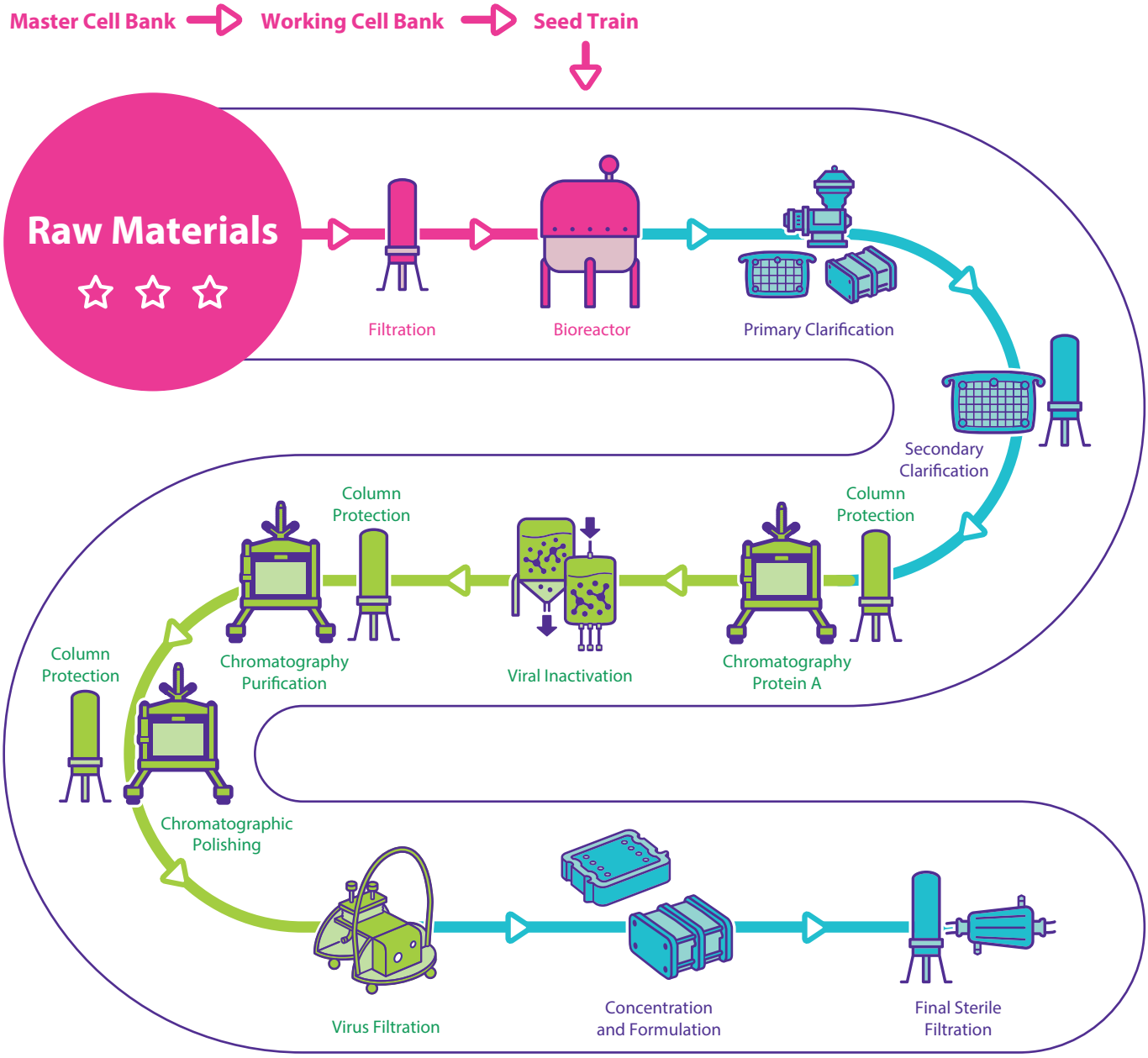
BioReliance® Biosafety Testing: cell line characterization, virus bank characterization, Next-Generation Sequencing, raw material testing, bulk lot and final product release testing

Support

Full suite of Emprove® buffers and process chemicals

Comprehensive support from initial process development through final implementation

Prevent, Detect, Remove:
Different Considerations for Upstream vs Downstream



Upstream
Prevent bioreactor contamination

Downstream
Demonstrate the process can remove virus

Centinel Intelligent Virus Defense™ Technology

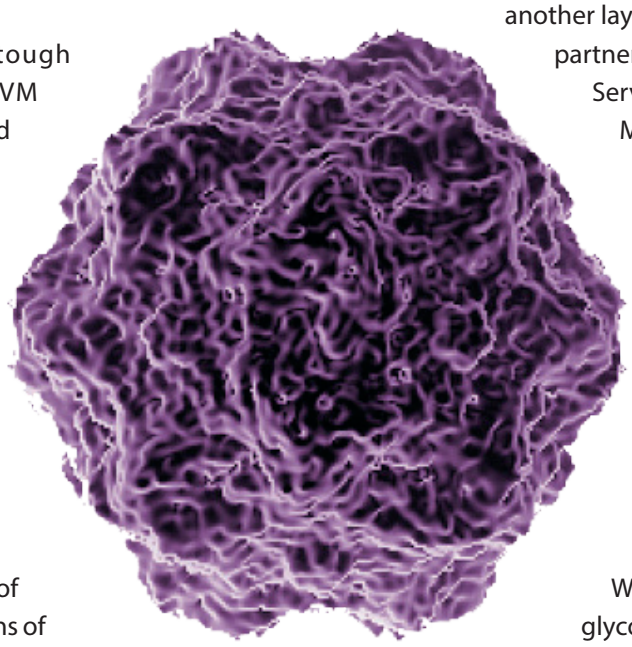
A New Line of Defense Against MVM Contamination

Powered by MilliporeSigma's advanced gene-editing platform, Centinel Intelligent Virus Defense™ technology represents the most significant advancement in viral safety since the removal of serum from manufacturing processes.

The Risk Of MVM Contamination

One of the major threats for manufacturers using CHO cells is minute virus of mice (MVM) contamination.

As it is so small, tough and heat-resistant, MVM is hard to remove, and contamination often goes undetected until a lack of productivity in CHO cell cultures is noticed in the bioreactor. MVM is highly virulent and specifically targets rapidly dividing cells; just one virus particle per liter can quickly take down a bioreactor full of CHO cells, costing millions of dollars. Regulators now expect manufacturers to test every bulk harvest for MVM. To demonstrate the virus safety of the manufacturing process, no virus should be detected. Now, there's a new way to guard cells against this pernicious viral threat.

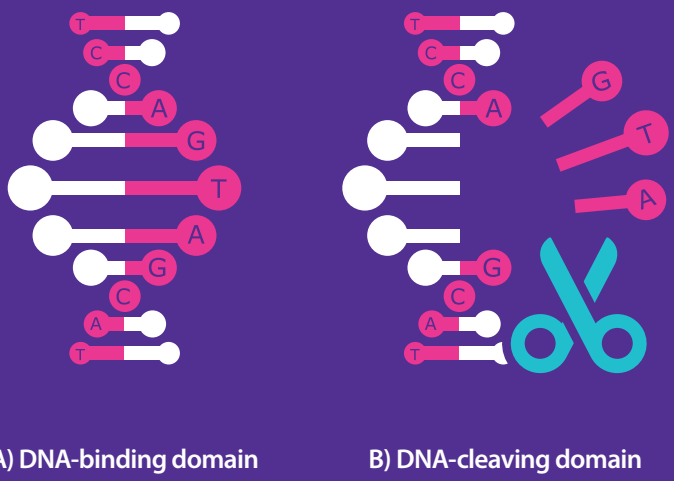


Minute virus of Mice (MVM)

Generate MVM-Resistant CHO Cells with Centinel™ Technology from MilliporeSigma

Centinel™ technology makes it possible to develop MVM-resistant CHO cell lines, giving manufacturers another layer of defense. Created in a partnership between BioReliance® Services viral safety experts and MilliporeSigma's cell-line gene editing team, Centinel™ technology represents an entirely new way to prevent viral contamination. Cell lines can be engineered using MilliporeSigma's gene editing technology, zinc finger nucleases (ZFNs), to suppress expression of sialic acid – a key component for MVM cell binding and entry. With no sialylated glycoproteins or glycolipids on the cell surface, MVM has no means of entering the cell, and so cannot replicate.

Extensive testing by BioReliance® Services team, both with lab strains and viruses isolated from biopharma producers, show that the cells are highly resistant to MVM, with no adverse effects on protein production. While the risk of viral contamination can never be totally ruled out, using cells that are MVM-resistant can benefit even the most thorough viral safety program.



What is ZFN Technology?

Zinc finger nucleases (ZFNs) are a class of engineered DNA-binding proteins that facilitate targeted editing of the genome by creating double-strand breaks in DNA at user-specified locations.

Centinel™ Technology: A Collaborative and Innovative Idea

If the receptor that MVM uses to gain entry could be eliminated, the virus may be unable to infect the cell.

"Viral safety is an issue that our customers – and the industry as a whole – take very seriously, and several major biopharmaceutical companies have championed efforts to improve viral safety in the industry," says the Centinel™ development team. "We developed Centinel™ technology in close collaboration with the industry and see it as another layer of protection in a company's overall viral mitigation program. After all, why take a risk you don't have to?"

Centinel™ Technology in Brief

A technology that provides security and sustainability by offering reagents, cell-line engineering services and viral challenge assays to promote viral resistance.

- No detectable MVM replication after viral challenge
- No drop in protein productivity or quality
- Suitable for production of asialylated recombinant proteins
- Centinel™ technology can be applied to multiple cell types and gene targets

More information: **www.Sial.com/centinel**

Millipore®
Filtration, Separation
& Preparation

robust.
productive.
proven.

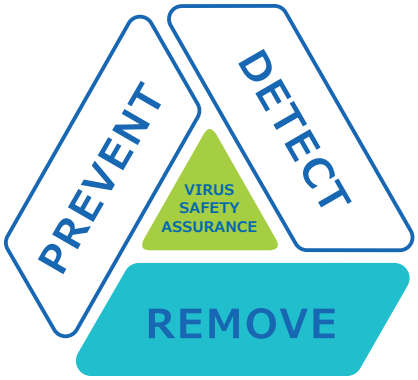
Viresolve® Pro Solution

When it comes to viral clearance, there is no room for error. You need products and services from a partner with a proven track record of success who understands your downstream viral clearance challenges.

Unmatched Viral Clearance
- Designed to handle the challenges of process interruptions
- 100% binary gas tested devices further enable high levels of virus removal

Unrivaled Productivity
- Optimized mass capacity with flexible prefiltration options
- High flux for faster processing
- Simplified setup and pre/post use IT

Market Experts in Downstream Viral Clearance
- Comprehensive support from initial PD and optimization through final validation and implementation
- Our BioReliance® services for virus validation studies



Viresolve®, a trusted name in viral clearance.

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The life science business of Merck KGaA, Darmstadt, Germany
operates as MilliporeSigma in the U.S. and Canada.

MilliporeSigma, 290 Concord Road, Billerica, MA 01821

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