New Paradigms in Viral Safety

With next generation bioprocessing and innovations in detection and clearance technologies it is time to rethink the traditional approaches to viral safety.

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viral safety risk Mitigation strategies

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A Biosafety Revolution

Biopharma manufacturers have been using the same assays for viral safety testing for decades, but new analytical technologies and molecular approaches offer a faster and more reliable approach. Until the next revolution...

By Afshin Sohrabi, Martin Wisher, and Audrey Chang

Monoclonal antibodies and other biopharmaceutical products, as well as their manufacturing processes, are inherently at risk of viral contamination, making viral safety testing critical. Viral safety testing is mandated by regulators worldwide, and although technologies for biomanufacturing have rapidly advanced, viral testing methods remain largely the same today as they were thirty years ago. Traditional virus detection approaches – cell-based assays – have served the biopharma industry very well over the years, but they have limitations; for example, some assays have long turn around times such as 28 days. In addition, although cell-based assays can detect contaminants, they generally cannot directly identify them and it can be slow to obtain results.

Albert Einstein once said, "Once we accept our limits, we go beyond them." In an age where speed is the key to success, we believe it is time to accept the limitations of traditional testing and to focus on newer technologies that focus on speed, sensitivity and reliability. Faster assay results will lead to more rapid batch disposition, reduced interruption of processing, and also meet the needs of more intensified processing – a key capability given the increasing interest that manufacturers are paying to continuous manufacturing strategies.

The molecular revolution

Although traditional assays remain the standard approach to biosafety and virus testing, biopharma manufacturers are increasingly being drawn to molecular methods such as broad specificity polymerase chain reaction (PCR) and next-generation sequencing (NGS), to expedite viral safety testing.

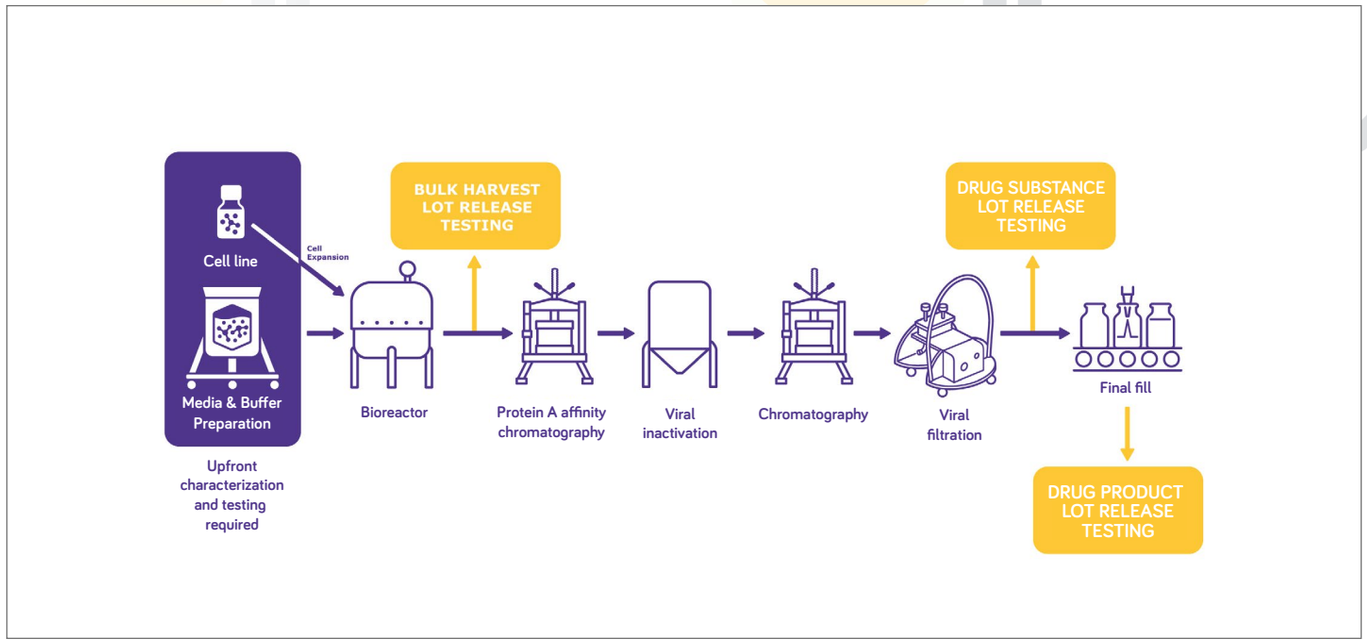

Figure 1: Lot release testing steps required for monoclonal antibody production.

NGS

Of all the molecular methods available, we think it's fair to say that NGS is the one that excites the industry. Many biopharma organizations employ NGS extensively in early stages of development for cell line characterization. Although the technology has been available for well over a decade, its use in biosafety testing is much more recent – and has only become feasible as sequencing costs have lowered and implementation methods have become standardized.

NGS is so effective as a molecular tool because it enables de novo identification of both known and unknown agents (viral, bacterial, or fungal) with precision and sensitivity. MilliporeSigma was the first to provide a GMP compliant NGS offering paired with a fully validated bioinformatics platform.However, despite these advantages, currently NGS tends to only be used where traditional testing approaches struggle or fail – for example where a product may be incompatible with cell-based viral detection approaches. However, for newer virus-based therapeutic products, where traditional assays are more challenging, NGS is an attractive alternative to meet virus testing requirements.

PCR

PCR enables detection of DNA or RNA sequences in vitro. It has been used in biosafety testing for the past twenty years, with the biggest advantages being that it is rapid (results available in a few hours) and highly sensitive. The largest issue with traditional PCR, however, is that small changes in the sequence of the target organism genome may result in a failure to amplify and potentially, a false negative result. The application of PCR in biosafety testing has evolved, with quantitative real-time PCR and, more recently, digital PCR approaches, allowing for more sensitive detection and more accurate quantitation of nucleic acid levels.

Other approaches are also enhancing the potential of PCR methods for virus testing. At MilliporeSigma, for example, we are working on broadening the detection capability of PCR by developing

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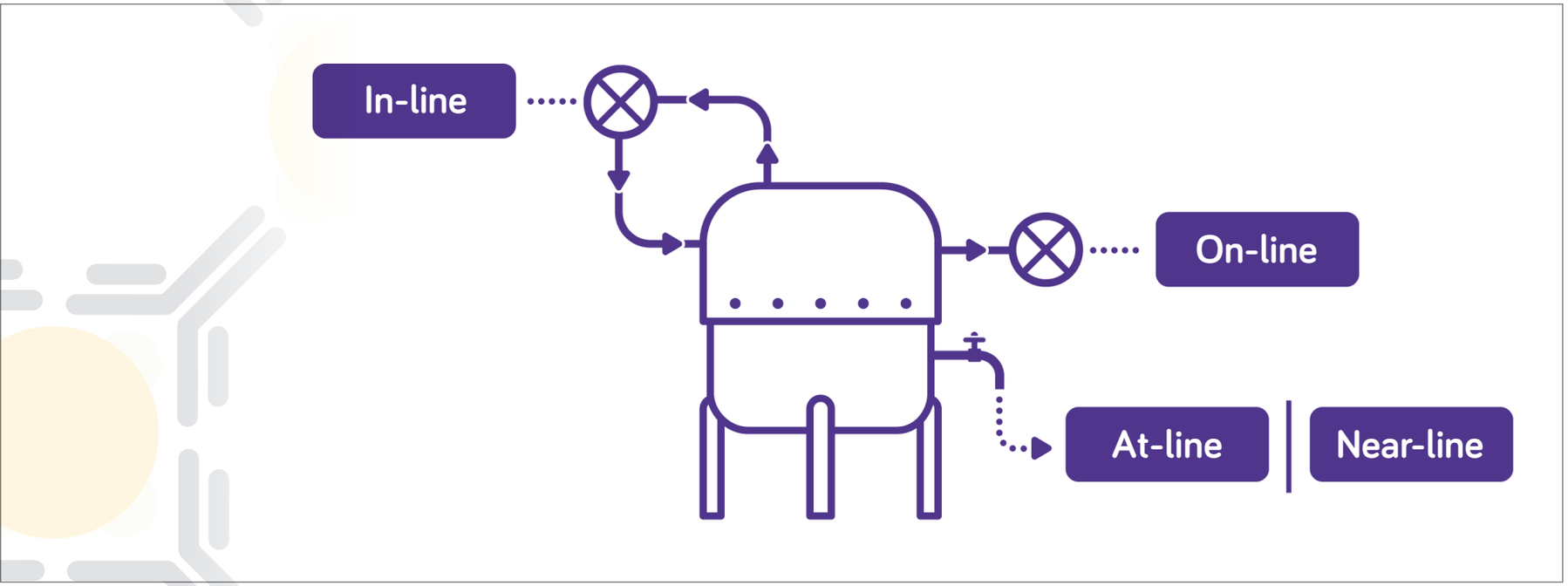


Figure 2: Diagram of the potential testing points from the bioreactor. In-line: bioreactor material constantly monitored. On-line: direct sampling of bioreactor for automated testing. At-line: sample removed for testing within manufacturing suite. Near-line: sample removed for testing within manufacturing facility.

degenerate primer sets that can broadly detect the seven families of DNA viruses and 14 families of RNA viruses relevant to CHO manufacturing. This novel approach using familiar technology enables us to identify a contaminant in a single test, rather than having to perform multiple different PCR tests. In our view, this expands the breath of detection while keeping the sensitivity and speed of PCR, opening up a huge opportunity to accelerate biosafety testing.

The next revolution

As both NGS and PCR methods evolve, they present clear opportunities to accelerate virus testing, which is meeting the needs of an industry that is looking for real-time decisions and information on the quality of the drug being manufactured. Indeed, these methods and other rapid testing technologies, such as biomonitoring and pyrogen detection, enable biopharmaceutical manufacturers to control their most important commodity – time.

A central conflict that we often see is that, although willing, biopharma manufacturers are often hesitant to implement new testing technologies due to concerns over regulatory implications. However, the regulatory documents on biosafety testing encourage the implementation of methods where it is demonstrated that the method is as good as, or better than, an existing technology; and that it meets the intended purpose of testing. The good news is that with the rise of cell and gene therapies, regulators are more frequently exposed to alternative and rapid testing strategies as traditional approaches are often not compatible with these modalities and the newer methods offer the only viable option for viral safety testing.

As any manufacturer will tell you, development of testing methods is only half the story. At MilliporeSigma, we are investing on the development of new biosafety methods and we also validate the performance of these tests to ensure they meet stringent GMP standards, thus bringing the confidence drug manufacturers need to use them.

Testing methods and approaches will continue to advance but the next revolution in viral safety testing may come sooner than we think. As biomanufacturing is moving to connected, continuous, intensified, and more automated processes, the notion that these highly developed manufacturing processes can wait for the time-to-results from traditional adventitious virus assays seems unlikely. The processes of tomorrow are looking for testing that can provide real-time test results enabling fast lot release, without compromising quality.

A current buzz in the industry is in-line testing, where testing is performed within the bioreactor environment for both ongoing monitoring as well as bulk harvest lot release. Realistically, not all technologies can be implemented this way and, to meet the needs of rapid time to results, some tests must evolve from being run in a testing lab away from the manufacturing site, to being able to be run close to the manufacturing line. We call this near-line testing. As the technologies develop, they can be brought ever closer to the manufacturing process, with testing on the manufacturing floor, or at-line. Our current thinking is that the closest these test technologies can get will be on-line, where a sample is taken from the process and consumed within a fully automated test. It is only with this evolutionary approach that virus testing timelines can reduce from days to hours, thus enabling intensified and ultimately continuous manufacturing processes (Figure 2).

Our teams of technical experts have been proudly supporting the biopharma industry for over 70 years with BioReliance® biosafety services and are committed to developing tests and services to support the evolving biologics market. Our experts understand the different needs of the processes that comprise drug product manufacturing and will work with you to design a solution that fits your needs.

Afshin Sohrabi, Ph.D, is Head of Near Real Time Testing Lab; Martin Wisher is Senior Regulatory Consultant; and Audrey Chang, Ph.D, is Head of PSS R&D. All are focusing on the BioReliance® biosafety portfolio at MilliporeSigma. The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the US and Canada.

The Holistic Approach to Upstream Viral Safety

There could be significant benefits to implementing additional measures in upstream bioprocesses to prevent viral contamination. But, given that upstream processes are complex, multiple strategies and technologies must be integrated within a holistic risk management program.

Craig Jackson and Michael Cunningham, Ph.D

When it comes to viral safety, biopharma companies have historically focused on their downstream processes, but recently there is increased awareness of the potential disruption viral contamination can bring, and a move towards mitigating the risk in upstream cell culture processes. The key driver for all aspects of viral safety is, of course, patient safety, and there are few regulatory pressures pushing companies to incorporate viral risk mitigation technologies upstream. Regulatory guidance focuses on ensuring cell lines and raw materials are well-characterized and free from detectable adventitious agents. However, there is a good business case to implement measures that minimize the risk of viral contamination and the potential disruption to manufacturing operations.

Where can contaminants enter the upstream process? Chinese hamster ovary (CHO) cells are especially susceptible to contamination with rodent viruses; minute virus of mice (MVM), in particular, has led to problems at a number of biopharma plants. Contamination often originates from raw materials and animal-derived components such as bovine serum or trypsin, which are regarded as particularly high-risk. Contamination can also originate from other cell culture components such as glucose – which attract rodents – or from equipment or facility operators, who can introduce human virus contaminants (such as adenovirus) into a process (Figure 1, viral risk identification).

Multiple lines of defence

The multitude of potential contamination sources necessitates a risk mitigation strategy built on complementary elements that prevent contamination and include both the raw materials and the manufacturing environment (Figure 2, viral risk mitigation strategies upstream).

A recently introduced option to reduce contamination risk is the availability of genetically modified CHO parental cell lines that have been engineered to eliminate the receptors used by the virus to enter cells, rendering them resistant to MVM infection. More traditional approaches for raw materials focus on sourcing, selection and treatment. Wherever possible, animal-derived cell culture components at high risk of virus contamination should be replaced with lower-risk alternatives, such as non-animal origin supplements, or recombinant proteins where the production processes reduce concerns about adventitious agent contamination. Where animal-derived components must be used, they should be carefully sourced from lower risk geographies. However, no material, even plant-derived or of recombinant origin, should be considered risk-free, as contamination may occur at any time throughout the supply chain of the raw material.

An additional virus risk mitigation option is to treat raw materials to inactivate potential viral contaminants before they enter the manufacturing facility - a "point-of-origin" strategy. For instance, bovine serum can be treated with gamma irradiation to inactivate viruses, and glucose can be treated with high-temperature short time (HTST) pasteurization to inactivate viruses – even those with high physico-chemical resistance. The recent availability of HTST-treated glucose manufactured under an ISO9001:2015 comprehensive quality management system is a new option for biomanufacturers, which may eliminate the need for significant capital investment and establishment of HTST technologies in-house.

In addition to "point-of-origin" approaches, the risk of viral contamination of cell culture processes can be reduced by implementing "point-of use" strategies that focus on treating materials immediately before they are used in the bioreactor. Filtration and HTST treatments of cell culture media are examples of point-of-use strategies that remove or inactivate potential adventitious organisms from cell culture media preparation. The

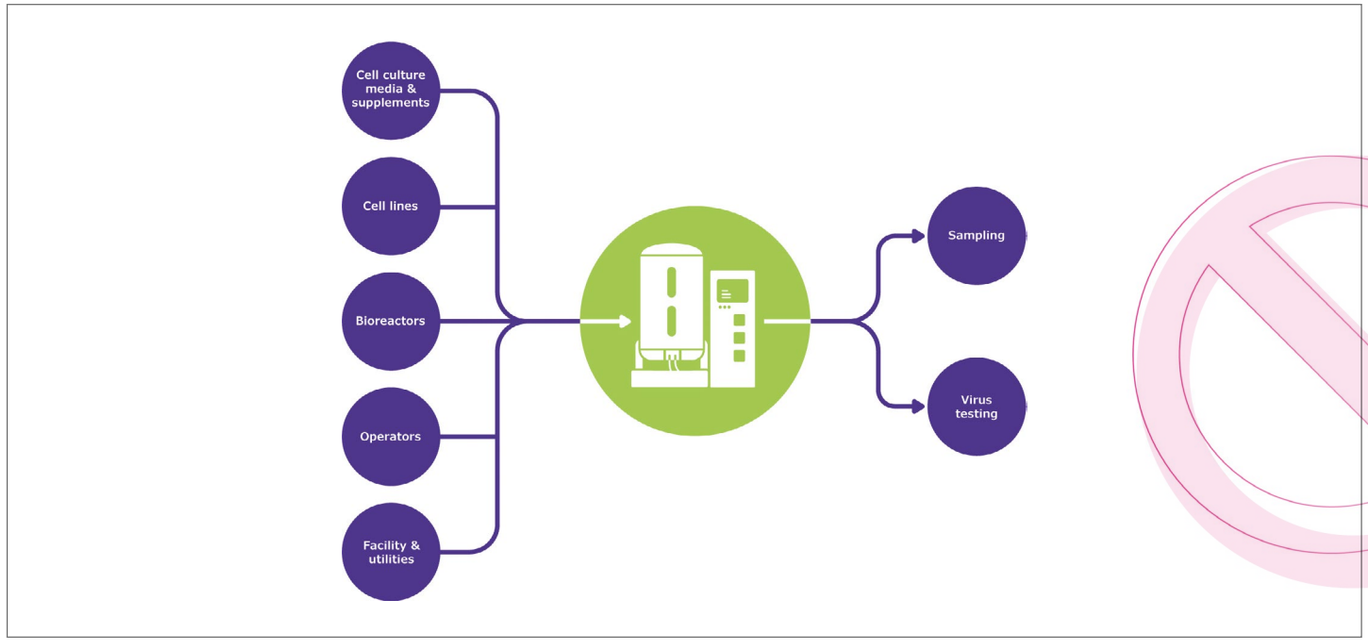
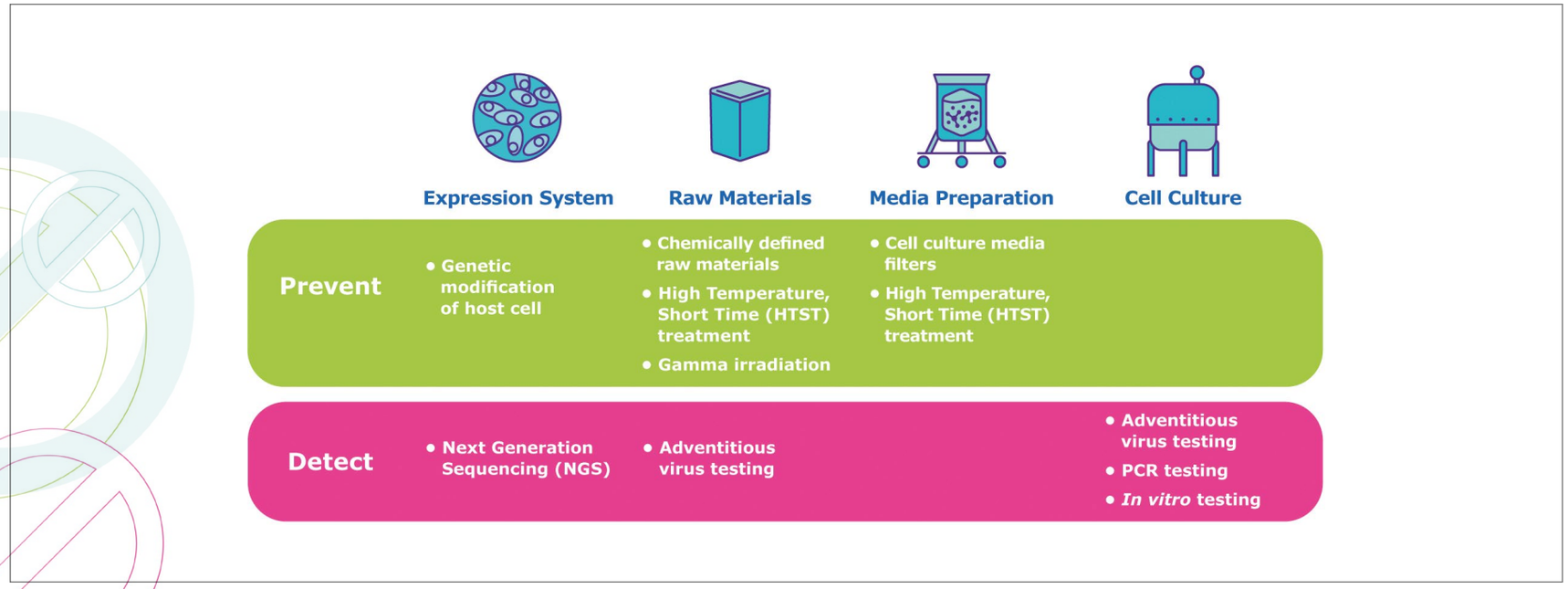


Figure 1: Viral risk identification is a prerequisite to a viral safety strategy.



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recent development of virus-retentive filters specifically designed to process cell culture media offer additional benefits to traditional sterilizing-grade filters for reducing the risk of introducing viral contaminants into the bioreactor. These filters efficiently process many different cell culture media, while delivering high retention of viruses and mycoplasma and sterilizing-grade performance for bacteria, all with low capital investment.

Perhaps the most effective approach to prevent contamination is to work with suppliers who follow high quality standards with transparency and visibility of their supply chain. Understanding the origin of raw materials and how they are controlled in a quality management system provides peace of mind.

The rise of real-time detection

Although there are several good options to reduce the likelihood of virus contamination, risk cannot be entirely eliminated. All comprehensive virus risk reduction strategies depend on a sensitive panel of virus assays capable of detecting contamination if it is present. Regulatory guidance documents provide a detailed framework of testing expectations for biologic products.

Traditional cell-based assays remain the standard approach to biosafety and viral testing, but biopharma manufacturers are increasingly being drawn to newer molecular methods, such as broad specificity polymerase chain reaction (PCR) and next generation sequencing (NGS), to expedite the viral safety testing process. These newer testing strategies can provide greater confidence in viral testing results and opportunities to accelerate testing.

Bioprocesses are also evolving – and connected, continuous, intensified, and more automated processes present some particular challenges to minimizing contamination risks of viral contamination. For example, the high media requirements for intensified processes increases the possibility of viral contamination. In addition, because steps are linked together, problems can ripple through the process – potentially to downstream operations. These challenges are best met with technologies that enable rapid, real-time monitoring of the upstream process.

Finally, it is also worth drawing attention to the advantages of implementing single-use technologies in upstream processes. Single-use technologies such as bioreactors, connectors and sampling devices are pre-sterilized with gamma irradiation before use, reducing the risk of introducing contaminants into the operation. In addition, if contamination occurs during processing, contaminated material and components can be rapidly disposed of. Single-use technologies eliminate much of the cleaning that might be required in the event of contamination in more traditional stainless-steel systems, enabling manufacturers to get back on-line faster. In an industry where time is money, single-use systems and components offer both flexibility and other advantages to manufacturers.

Staying safe

Traditionally, preventing upstream viral contamination has focused on sourcing and testing raw materials. This approach to risk mitigation has worked well for many years and, to date, no contaminated biopharma products have reached a patient. For manufacturers, however, viral contamination events are incredibly disruptive and expensive; high-profile cases are driving companies to re-examine their risk assessments around virus safety and take steps to reduce risk upstream of the bioreactor.

There is no single viral safety solution that works for every process – solutions depend on the process, media components, facility, scale, and the type of biologic being produced. Multiple strategies and technologies to mitigate risks should be integrated into an overall virus safety management program – based on effective risk analyses. In short, a holistic approach that considers all components of the manufacturer's process, is the only reasonable approach to minimize pathogen safety risk.

Craig Jackson is Senior Account Manager, and Michael Cunningham, PhD, is Associate Director, Upstream Global MSAT, both at MilliporeSigma.

Next Generation Bioprocessing and the Implications for Viral Safety

With many companies embracing the move to next generation bioprocessing, it is important that they do not forget to re-examine their approach to viral safety

By Kathy Remington, Ph.D, and Michael Phillips, Ph.D

Biomanufacturers are heralding next generation bioprocessing as a way to improve efficiency and productivity, reduce plant footprint and operating costs while maintaining the highest quality standards for the therapies being produced. Compared to traditional batch processing, this new manufacturing paradigm may include higher concentration fluids, higher mass loading of individual unit operations, longer duration processes and connected or continuous processes.

However, maximizing the benefits of this new approach to manufacturing requires consideration of the entire process from a holistic perspective. For example, process intensification using perfusion methodologies results in high density cell cultures that maximize protein productivity in relatively small bioreactors. While this can compress upstream timelines and increase protein yields per unit volume from the bioreactor, downstream operations may struggle to keep pace with higher titers from improved upstream operations.

It is clear that from a process development perspective, we need to consider the implications of efficiency improvements in a single operation in the context of the overall process. Overlaid on this, we may need to rethink how these changes might impact viral safety and how we assess the clearance capabilities of the individual operations.

Viral safety considerations with intensified processing

Higher concentration process intermediates, higher mass loadings on individual operations, longer duration processing, and connected or continuous processing all have the potential to impact viral safety.

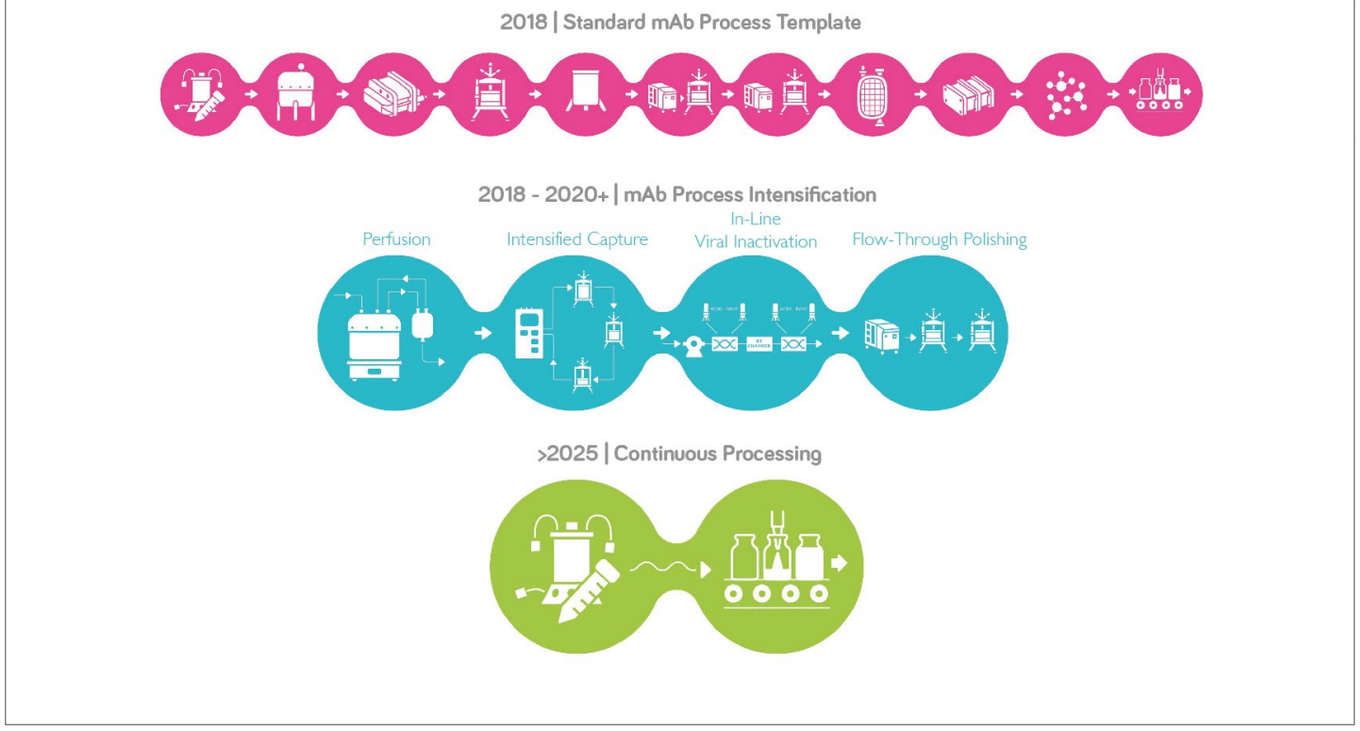


Figure 1: Viral safety strategies need to be re- examined as the mAb production template evolves to next generation bioprocessing.

- **Higher concentration processing.**

  High protein concentrations could impact virus inactivation – either through changing the buffering conditions for low pH virus inactivation or potentially interfering with viral inactivation using detergents. In the latter case, as long as the concentration of detergent or solvent/detergent is maintained, the higher protein concentration is less likely to impact inactivation.
  Highly concentrated loads may impact the performance of chromatography and filtration steps. Whether the chromatography step is run in bind and elute or flow-through mode, and regardless of the type of resin or membrane, the higher concentration of process intermediate, and potentially impurities, could influence the efficiency of the chromatographic separation. Viral clearance across the step may also be impacted through non-specific interactions of virus with the high concentration intermediate and the chromatographic resin, which may result in more virus binding to the intermediate or resin and consequently lower viral clearance. Similarly, higher concentration intermediates may impact virus filtration, necessitating increased use of prefilters

to remove protein aggregates, or additional filtration membrane area. To confirm viral safety targets are met across downstream unit operations, viral clearance studies should be performed with higher concentration load solutions.

- **Higher mass loadings.**

  With intensified processing, a major goal is to identify technologies that offer high productivity, processing the same amount of mass through much smaller devices. For the most part, downstream processing of higher concentrations is advantageous, resulting in smaller intermediate product hold tanks, decreased loading times onto chromatography resins, and potentially higher effective capacities during chromatographic operations – all key advantages of the approach. The biggest concerns would be potential competitive binding, which could reduce separation efficiency, and potentially introduce issues with protein stability. Chromatography resins and membranes for intensified processes should be capable of operating at high mass loadings while maintaining the expected separation resolution. For virus filtration, high mass loading of high concentration feeds could require more membrane area, unless the capacity of the virus filter can be increased. In addition, mimicking the at-scale process in a clearance evaluation would require a significant mass of product for small scale tests, and there is a higher likelihood that the virus spike itself might interact with the high concentration feed, which could, in turn, affect the filterability of the process solution.

- **Longer duration processing.**

  Intensified processing may involve targeting the same mass loading, but operating at lower flux for a longer duration. Depending on process duration, this is generally not expected to impact viral safety. Clearance evaluations would need to mimic this scenario, and include several starts/stops or process interruptions to mimic likely processing conditions. In addition, after several days of processing, bioburden could be a concern so manufacturers may need to think differently about bioburden control.

- **Connected/continuous processing.**

  Adoption of this strategy may have the biggest impact on viral clearance assessments as there will most likely be two unit operations running simultaneously both of which are designed to remove virus; for example, anion exchange chromatography and virus filtration. During a standard batch process, it is easy to isolate process steps and evaluate the viral clearance potential of each step independently. For a continuous process, it is more difficult to isolate each step, and instead of assessing clearance of a homogeneous batch, clearance would be evaluated across a step where the load solution might have a different composition at the start and the end of the 'batch'. Additionally, evaluation of viral clearance will require specialized techniques and equipment.

Importantly, if an existing process for which viral clearance data have previously been generated is modified and intensified, that clearance data may no longer be valid. The increased concentration of the process intermediate, adjusted loadings on individual unit operations and slight modifications to the unit operation process window may impact the levels of viral clearance that can be achieved for individual steps. To assure the new process can deliver the expected level of viral safety, clearance studies should be performed using the new, more concentrated intermediate under the new process conditions.

Evolving the approach to viral safety

It is clear that next generation bioprocessing strategies impact the approach to viral safety. By connecting process steps, we can no longer evaluate the viral clearance of isolated steps, and process development should ideally include viral clearance evaluations. In addition, unit operations may be impacted by the previous step and the load solution to a step may not be homogeneous. This will require a creative approach to modeling newly developed processes to ensure they accurately represent the manufacturing operations. Furthermore, how we execute virus spiking studies may need to be re-evaluated to minimize any negative impact of addition of virus to the test system.

At MilliporeSigma, we are focused on enabling advanced manufacturing through our BioContinuum™ Platform strategy that includes new process technologies and systems combined with new digital solutions. From a process technologies and systems perspective, we are developing solutions to support intensified fed-batch and perfusion processes, intensified capture, in-line viral inactivation, integrated flow through polishing, and continuous ultrafiltration/diafiltration. From a digital perspective, we are developing a new control platform and orchestration platforms that would be 'future-ready' to support additional digital technologies required to enable advanced manufacturing.

The way forward

From a viral safety perspective, monoclonal antibodies and recombinant proteins have a very safe track record. Although we may feel confident that next generation approaches are similarly safe, we need to demonstrate that intensified and continuous processes deliver the expected levels of viral safety. Doing this with confidence will require creativity in the development of novel spiking strategies and accurate small-scale models that reflect new processing conditions.

Undoubtedly, intensified processing requires the biopharmaceutical industry to think differently and more holistically. The ultimate benefits of the adoption of next generation approaches, however, far outweigh any challenges presented by technical, regulatory, and implementation aspects.

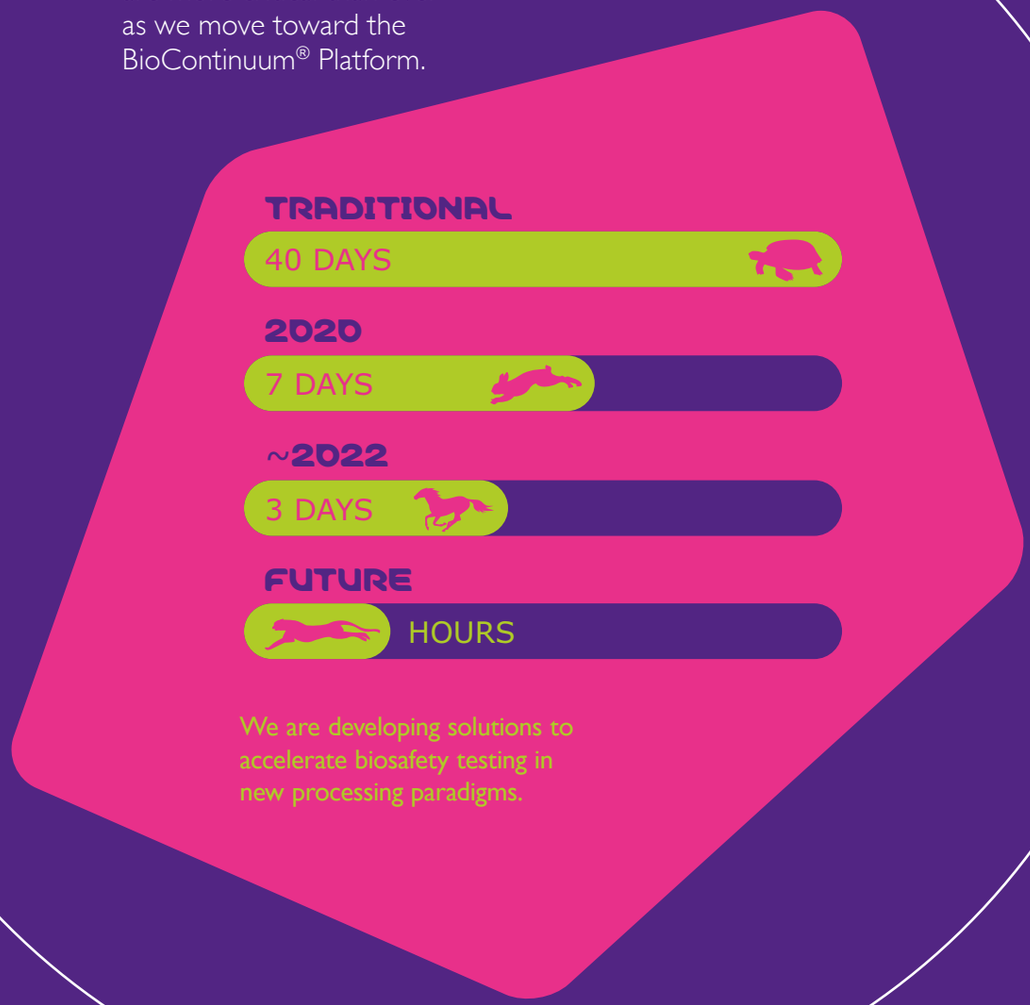
Kathy Remington is a Technical Consultant focusing on the BioReliance® portfolio, and Michael Phillips is Director of Next Generation Bioprocessing R&D, both at MilliporeSigma.

Alternatives to *in vivo* Assays for Biosafety Testing of Biologics

The use of animal models for the detection of adventitious agents has been a feature of biologic testing packages for many decades. However, as alternative methods such as PCR and NGS have emerged these *in vivo* tests have stubbornly remained a central part of testing. Here, we examine the current *in vivo* methods and explore alternatives which can be employed today. We also propose that while the industry may be some years away from removing *in vivo* testing completely, a case could be made for removing animal use from very well-characterized production systems such as CHO.

View the full application note online

Rapid detection technologies are more critical than ever as we move toward the BioContinuum® Platform.



We are developing solutions to accelerate biosafety testing in new processing paradigms.

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BioContinuum™ Platform for Next Generation Bioprocessing

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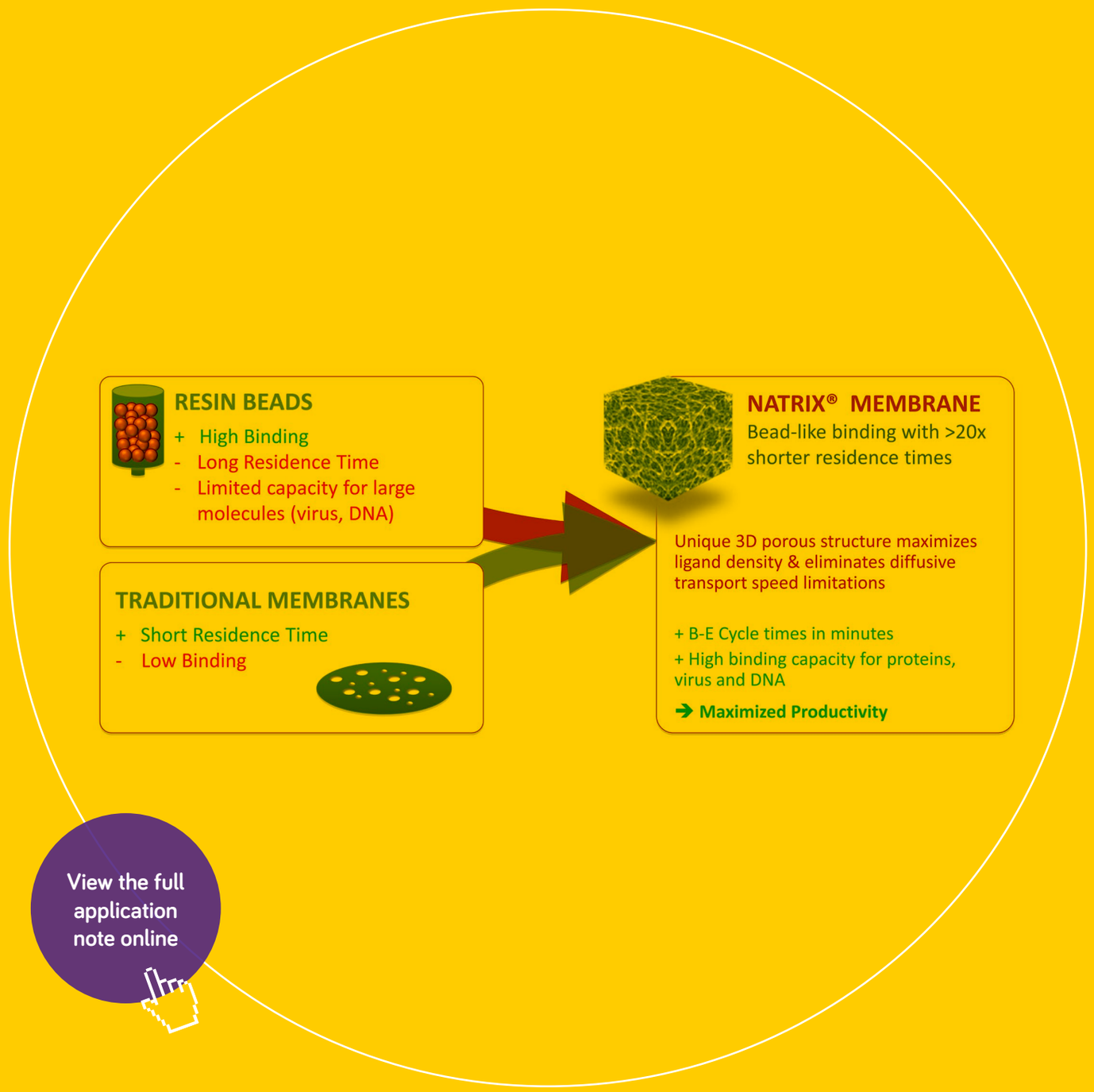
Robust Viral Clearance in Monoclonal Antibody Purification with NatriFlo® HD-Q Membrane Adsorber

Increased process efficiency and excellent viral clearance.

Anion exchange chromatography is an important flowthrough polishing step for monoclonal antibodies (mAbs). Chromatographic resins are most commonly used for this step, but the long residence time requirement due to diffusion mass transfer and large size of virus particles limits process throughput and binding capacity, resulting in oversized resin volume and associated supporting hardware and facility footprint. Moreover, in clinical manufacturing where the oversized column is used for only a fraction of its usable life, the path to realize cost efficiency is challenging: multi-use column chromatography steps incur high operating costs for routine cleaning and storage as well as validation.

The high ligand density and open structure of the NatriFlo® HD-Q membrane adsorber avoids the limitations of resin-based materials by offering a high-capacity, high throughput single-use option for flow-through polishing applications. This innovative technology offers increased process efficiency while delivering excellent viral clearance performance. In this study, viral clearance of a panel of viruses was assessed across a range of conditions of different pH, conductivity, and buffers.



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