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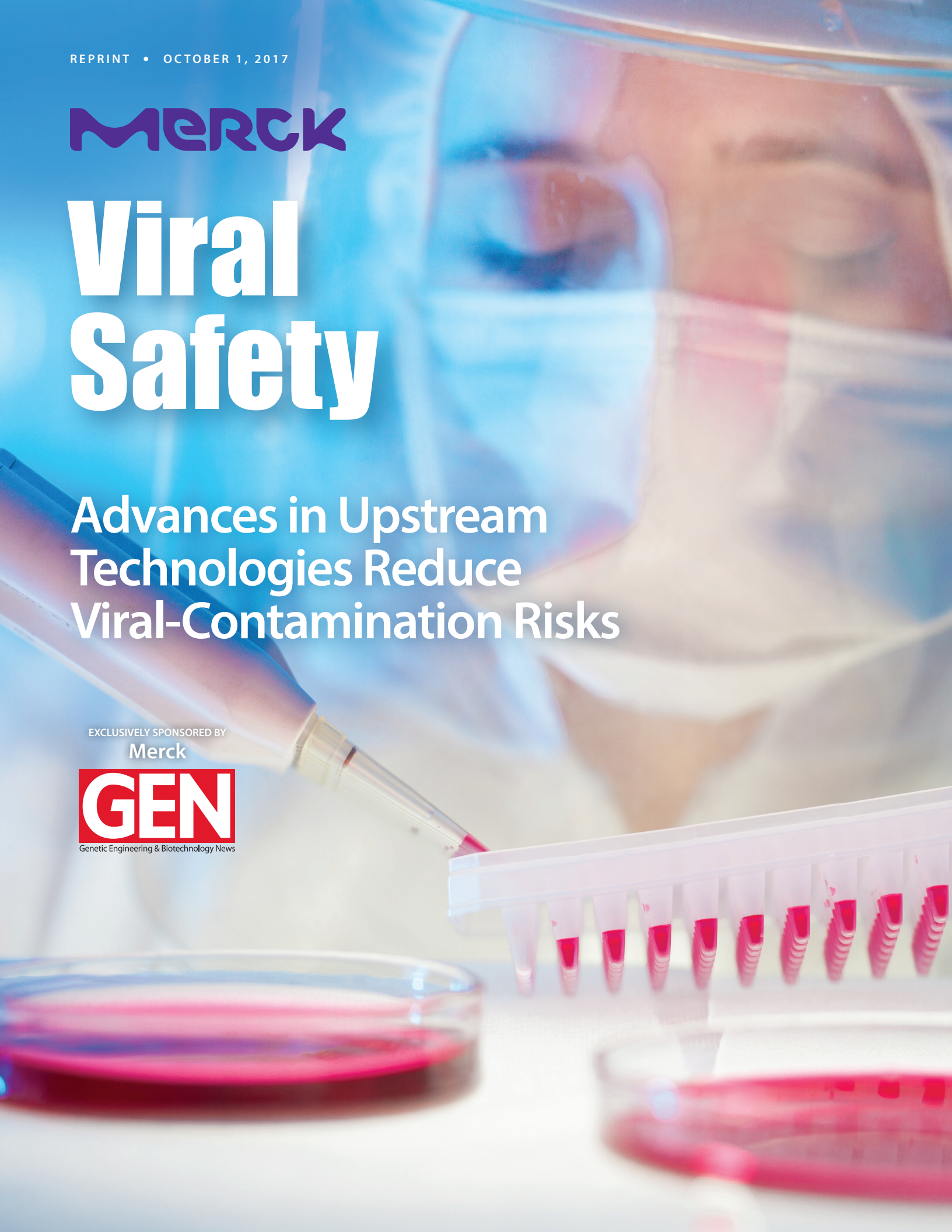
Viral Safety

Advances in Upstream Technologies Reduce Viral-Contamination Risks



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

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

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.

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.

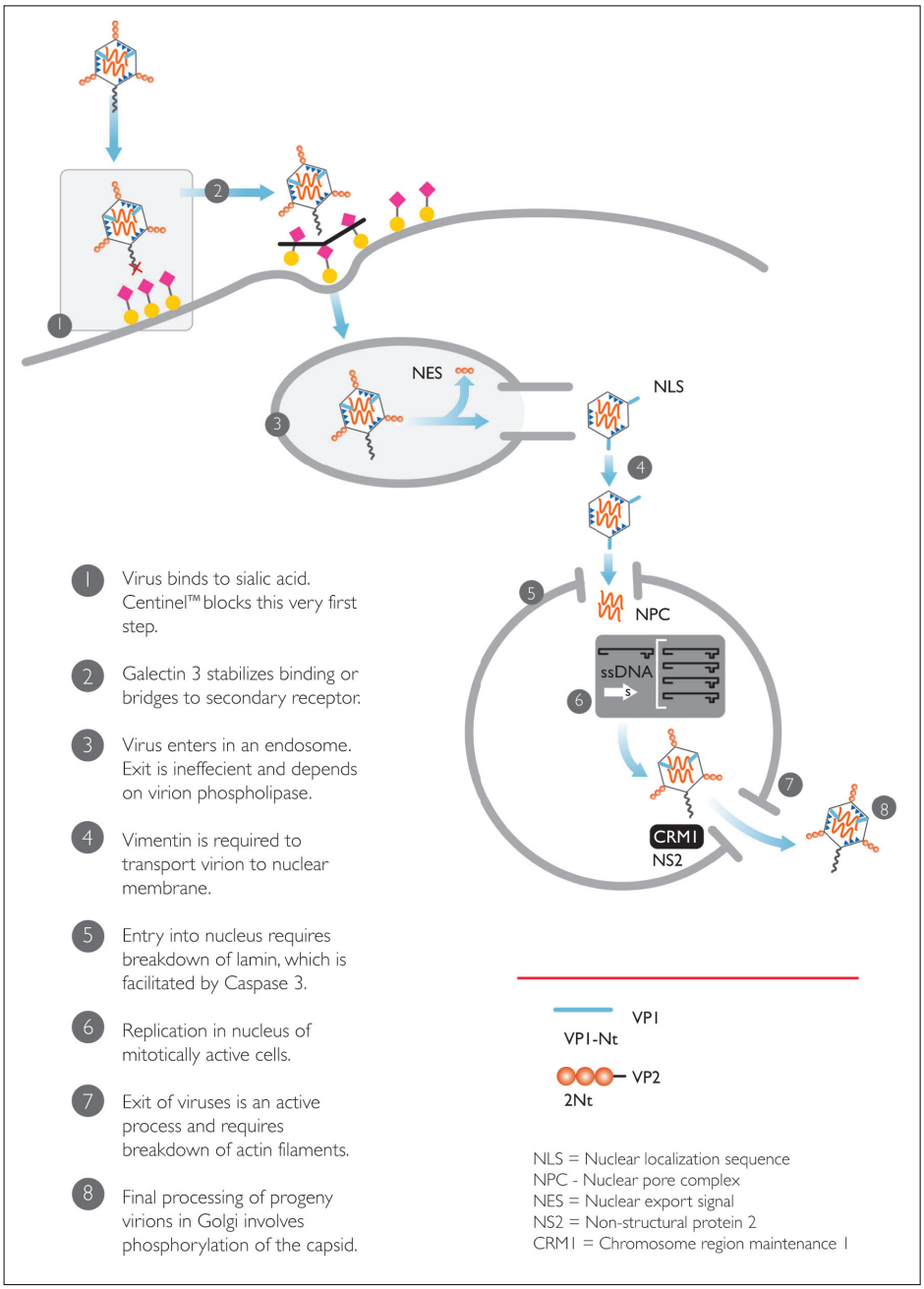
Ensuring Product Quality

"Companies need to implement a multilayered approach for their viral risk mitigation strategy," explained Kevin Kayser Ph.D., head of upstream R&D at Merck. "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



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

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.

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

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

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 Merck. "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.

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. ■

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. ■