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**Prachi Khekare**

**Justin Ozeki**

**Ryan Lindquist**

**Hamada Aboubakr,  
MSc., Ph.D.**

**Brandon Johnson,  
Ph.D., MLS(ASCP)CM**

Microbiologics

# Mitigating adventitious agent risks in CGT

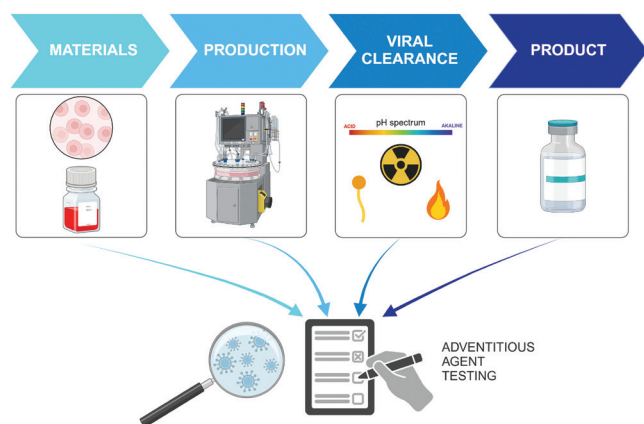
## Innovations in detection and control

■ The concept of gene transfer to modify the genome for disease treatment began emerging in the latter half of the 20th century, with significant progress in the 1970s and 1980s. In 1968, the first successful bone marrow transplant was performed to treat severe combined immunodeficiency, demonstrating the potential of cell therapy. In 1990, the first approved gene therapy trial was conducted for severe combined immunodeficiency, marking the beginning of the cell and gene therapy (CGT) era (Wirth et al., 2013; Heelan 2023).

CGTs refer to advanced therapeutics that utilize modified living cells or genetic material products to replace, repair, or enhance the biological functions, and offer potential cures for previously untreatable conditions. Cell therapy and gene therapy are closely related and overlapping fields, differing in their definitions, techniques, and the types of biomaterials administered to patients. Cell therapy involves the transplantation or manipulation of live cells to restore, replace, or enhance biological functions. This includes hematopoietic stem cell (HSC) transplantation, which has been widely used for treating hematologic malignancies such as leukemia (Appelbaum, 2007), and chimeric antigen receptor (CAR) T-cell therapy, which modifies a patient's T cells to recognize and attack cancer cells, demonstrating remarkable success in B-cell lymphoma (June et al., 2018). The primary biomaterials administered in cell therapy are living cells, either autologous or allogeneic, often expanded or genetically modified *ex vivo* before infusion (Mason & Dunnill, 2009).

On the other hand, gene therapy focuses on introducing, modifying, or repairing genetic material within a patient's cells to correct disease-causing mutations or introduce therapeutic functions. Gene delivery techniques such as electroporation, direct injection, viral vector-mediated gene delivery (e.g., lentiviral or adeno-associated virus), lipid nanoparticles (LNPs), and gene editing tools like CRISPR-Cas9 enable precise genetic modifications (Naldini, 2015; Sayed et al., 2022). Gene therapy has successfully treated spinal muscular atrophy using AAV-based delivery of the SMN1 gene, as seen in the FDA-approved drug Zolgensma (Mendell et al., 2017), and has been used to treat hemophilia B (factor IX deficiency) through gene transfer strategies that restore clotting factor production (High & Roncarolo, 2019; Chowdary et al., 2022). The biomaterials used in gene therapy are typically genetic constructs, such as plasmids, RNA, or viral vectors designed to modify cellular function.

The worldwide increase in the need for CGT to treat a variety of genetic disorders has led to a surge of more refined investigations in this field, along with some products receiving regulatory approvals. In 2024 alone, seven new CGTs received U.S. FDA approval, increasing the number of FDA approved CGT products to 43 as of December 2024. This indicates the remarkable success of CGT in treating previously incurable diseases. However, producing and administering these therapies present significant safety concerns and challenges, including insertional mutagenesis, anti-AAV host immune response, editing inaccuracy (e.g. off-target mutations), and the risk of adventitious agent contamination—the main focus of this editorial—which can compromise product safety and potency of the CGT products (Tang & Xu, 2020; Aranha, 2023). Adventitious agents, such as viruses, bacteria, mycoplasma, and other microorganisms, pose a serious threat to CGT products. These contaminants can originate from raw materials, cell cultures, or the manufacturing process, potentially leading to severe patient harm or product recalls (Chawla et al., 2016; Philippidis, 2020) (Figure1).



**Figure 1.** Adventitious agents can be introduced at multiple points in the manufacturing process, therefore adventitious agent testing is required at several steps in the product manufacturing process.

Traditionally, the detection of adventitious agents has relied on a combination of *in vivo* and *in vitro* assays. Animal-based tests, such as mouse and guinea pig assays, have been used to detect viral and bacterial contaminants, while cell culture methods and electron microscopy have been employed for broader pathogen screening (Gombold et al., 2014; Aranha, 2023). While these methods have been the gold standard for decades, they are often time-consuming, labor-intensive, and ethically contentious. Moreover, they may lack the sensitivity and specificity required to detect low-level or emerging contaminants, highlighting the need for more advanced and efficient detection technologies. In response to these limitations, regulatory agencies such as the FDA have encouraged a shift away from *in vivo* testing toward more innovative, high-throughput, and ethical alternatives (FDA Modernization Act 2.0). This regulatory transition aligns with the broader movement advocating for the reduction, refinement, and replacement of animal testing, driving the adoption of modern methods for adventitious agent detection (Gorzalczyk & Rodriguez Basso, 2021). Advanced molecular techniques, including next-generation sequencing (NGS), digital PCR, and mass spectrometry-based proteomics, have emerged as powerful tools for ensuring the safety of CGT products (Qiu et al., 2013, Flatschart et al., 2015; Khan et al., 2017).

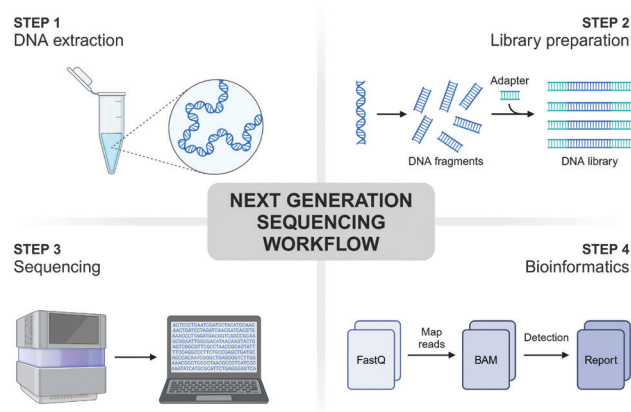
## The genomic revolution of adventitious agent testing

NGS is an emerging tool for detecting adventitious agents, offering comprehensive, broad-spectrum screening and highly sensitive detection methods. Since its introduction in the mid-2000s, significant advancements have been made to lower costs, increase throughput, and improve read quality and overall turnaround time. These improvements have made genomic data accessible for diverse applications (Goodwin et al., 2016; McCombie et al., 2019). NGS provides the advantage of highly specific genomic discrimination through mass parallelization, allowing millions of sequences to be read simultaneously.

This capability offers a distinct advantage, as viral nucleic acids are sequenced alongside the background host material.

By capturing broad genetic information from a sample, NGS enables the detection of more than just a limited number of target viruses typically identified by conventional methods. This method is agnostic to the viral genome and physio-chemical properties, making NGS an appealing advanced detection technology for viral safety testing (Khan et al., 2017; Sathiamoorthy et al., 2018). In light of these advancements, the most recent updates to the ICH Q5A (R2) guidance emphasize the adoption of NGS to supplement *in vitro* testing and potentially replace *in vivo* testing (ICH, 2023; Hirai et al., 2024). Moreover, the adoption of this technology to replace *in vivo* testing supports the 3Rs initiatives—replacement, reduction and refinement of animals for testing (EDQM, 2023). There are many applications for NGS technology in the detection of adventitious agents in biologics. As a material screening tool, NGS provides a means to assess materials to prevent introduction of endogenous virus. In-process testing with NGS enhances the detection of unexpected contaminants introduced during manufacturing. As a part of viral clearance, NGS testing can evaluate the efficacy of filtration methods for virus removal from products.

The process of NGS testing consists of upstream sample handling, library preparation, sequencing, and downstream bioinformatics, adding to the complexity of evaluating assay performance (Figure 2). While NGS technology is highly versatile, the considerations for evaluation are unique to the lab, instrumentation, and intended application, making standardization challenging. The type of testing material may necessitate the use of unique approaches, such as methods for genomics and transcriptomics. Targeted methods for NGS using amplicon sequencing and hybridization capture may also be considered for detecting known potential contaminants. Unique testing modalities may require different processing steps, leading to performance variations based on the assay's methods. For applications in the detection of adventitious viruses, it is critical that each assay be tested with a diverse set of viruses representing various physiochemical and genomic properties of virus families to properly assess assay specificity, limit of detection, and the impact of product-specific matrices.



**Figure 2.** Schematic of the four primary steps in an NGS workflow. (2022) BioRender.

Perhaps the most challenging element of detecting adventitious agents with NGS is the evaluation of millions or billions of sequencing reads to detect the needle in the genetic haystack. This process requires the implementation of a purpose-built bioinformatics workflow to inspect the large number of data points. This workflow includes the evaluation of sequence reads for both quality and identity, including a process for read mapping and screening against a reference database for the detection and identification of adventitious agents. The quality of the reads and the database used for reference is critical for the optimal performance of the analysis. In an effort to produce a comprehensive repository of viral, virus-related, and virus-like sequences, the Reference Viral Database (RVDB) was produced. It is publicly available and updated regularly with both a comprehensive unclustered database and a clustered database that reduces redundancy at 98% identity (Goodacre et al., 2018).













However, as this is a relatively new and inherently complex technology, there are both technical and regulatory challenges with implementing NGS testing methods for adventitious virus safety testing. Without established and standardized implementation strategies, unique testing requirements and variations in analysis pipelines, as well as wet lab assays, complicate regulatory oversight. Further, adopting a new technology as a replacement for established methods requires a clear and concise explanation of the relevant benefits. Early discussions with the relevant health authorities may facilitate scientific advice regarding the unique requirements for the intended application, streamlining communication on questions and concerns as well as detailing expectations for planned studies (EFPIA, 2024). The regulatory framework continues to evolve, with specific recommendations recently outlined by the ICH Q5A. Additionally, organizations such as the European Medicines Agency (EMA), the European Directorate for the Quality of Medicines & Healthcare (EDQM), and the U.S. FDA provide insights for selecting the best solutions to align with testing requirements. Increased familiarity with and guidance on best practices are rapidly advancing the acceptance of NGS technology for adventitious agent testing and the worldwide adoption of innovative and advanced technologies.

## Standard biomaterial controls for HTS-based assay validation

The reliability of high-throughput sequencing (HTS) assays depends on performing rigorous validation using well-characterized control virus biomaterials. These controls are essential for assessing the sensitivity, specificity, and reproducibility of sequencing workflows. Incorporating known viral sequences into test samples allows researchers to systematically evaluate HTS performance, ensuring accurate detection of both known and novel contaminants (Rong et al., 2023). Moreover, control virus biomaterials facilitate the standardization of HTS methods across laboratories and regulatory agencies, promoting consistent quality control and compliance with good manufacturing practices (GMP). They are critical for benchmarking bioinformatics pipelines, minimizing false positives and negatives, and optimizing sample preparation protocols. Their use also supports regulatory

submissions by providing evidence of assay robustness and detection limits (Khan et al., 2024).

The launch of the first WHO International Reference Panel for adventitious virus detection in biological products marked a significant milestone in the standardization and validation of HTS and other molecular methods used for viral safety testing in gene and cell therapies (Khan et al., 2024). This panel was developed to incorporate a globally recognized set of well-characterized viral reference materials, representing different genome types (RNA and DNA viruses) and different virus structures (i.e., enveloped and non-enveloped) (Figure 3).

GROUP	EXAMPLE	GENOME
GROUP 1 ds DNA	 -Epstein-Barr virus	 dsDNA
GROUP 2 +ssDNA	 -Porcine Circovirus -Minute Virus of Mice	 +ssDNA
GROUP 3 +dsRNA	 -Orthoreovirus	 dsRNA
GROUP 4 +ssRNA	 -Betacoronavirus	 +ssRNA
GROUP 5 -ssRNA	 -Respiratory Syncytial Virus	 -ssRNA
GROUP 6 +ssRNA-RT	 -Feline Leukemia Virus	 +ssRNA

**Figure 3. Baltimore group classification and genome structure of diverse viruses available in the WHO International Reference Panel for Adventitious Virus Detection. Modified from BioRender (2021).**

The WHO panel has played a crucial role in standardizing adventitious virus detection by HTS. However, opportunities for further enhancements remain. One area for improvement we are addressing at Microbiologics is expanding the viral diversity of a similar list that we have been developing, as the WHO panel currently includes only a selected set of seven viruses. This short list may not fully represent all potential adventitious agents in biological products listed in the RVDB, developed by Dr. Arifa Khan's group at the Center for Biological Evaluation and Research (CBER) (Goodacre et al., 2018). Furthermore, improving cost and accessibility could help more laboratories, particularly smaller research facilities or those in resource-limited settings, benefit from these standardized materials. Despite these challenges, the panel remains a vital resource for strengthening viral safety testing and regulatory compliance in CGT. Another important consideration is the need for customized adventitious virus reference materials that include specific viral controls at defined concentrations or precise mixing ratios to meet the needs of some validation studies. Additionally, the limited lot size of the WHO panel raises concerns about limited accessibility and lot-to-lot inconsistency moving forward.

To address the areas for improvement outlined above, reference biomaterial manufacturers such as Microbiologics have identified both challenges and opportunities in developing high-quality adventitious virus reference materials. One primary challenge is the procurement of specific adventitious viruses, which may be difficult to source due to regulatory restrictions, limited availability, or commercialization constraints. To overcome this, manufacturers must establish strategic partnerships with research labs, biobanks, veterinary institutions, or field surveillance programs capable of providing animal-derived or field samples that test positive for the target adventitious viruses. These collaborations must comply with all necessary commercialization licensure agreements, regulatory approvals, and associated fees. Once sourced, manufacturers must undertake rigorous processes for virus isolation, purification, and characterization to ensure the production of well-defined reference materials. Additionally, optimizing and scaling up virus propagation methods is essential to generate sufficient quantities of high-quality viral stocks for standardization and widespread distribution.

Hard-to-culture, unculturable, and unprocurable viruses—whether due to scarcity or regulatory constraints—pose significant sourcing challenges. However, synthetic virology and modern reverse genetics offer efficient alternatives for reference biomaterial manufacturers, despite inherent risks and uncertainties associated with this approach. Traditional reverse genetics, reliant on clone-based methods for constructing infectious clones of the virus genome, is time-consuming, low-throughput, and requires infectious virus isolation. In contrast, modern approaches leverage *de novo* synthesis of full-length viral genomes, once costly and complex but now more accessible due to advancements in DNA synthesis chemistry, instrumentation, and enhanced gene assembly techniques for large DNA constructs, along with enzymatic error-correction methods. Enhanced bioinformatics tools and extensive publicly available genomic databases further streamline these processes, making synthetic virology more cost-effective, time-efficient, high-throughput, error-free, and independent of clinical virus samples. Furthermore, using *de novo* synthesis and well-defined genetic constructs in synthetic virology techniques enables precise control over viral genome synthesis, ensuring consistency in virus specifications across production lots and helping to avoid genomic drift over passaging when traditional techniques of viral isolation and propagation are used. In line with this approach, we developed state-of-the-art *de novo* viral genome synthesis and synthetic virology capabilities and successfully established a synthetic virology system for recovering retroviruses and polyomaviruses. This serves as an initial demonstration of our ability to apply similar methods for the synthetic recovery of animal retroviruses, supporting adventitious agent research (Li et al., 2024).

## Purification and concentration of biomaterial controls

Ensuring the safety and efficacy of CGT products requires not only robust detection of adventitious agents but also effective

purification strategies that physically remove contaminants. Adventitious agents pose significant risks to CGTs, potentially triggering immune responses and compromising regulatory approval. Stringent purification is therefore required to meet global regulatory standards set by the FDA and the EMA. To achieve this, CGT manufacturers employ a combination of filtration, chromatography, and ultracentrifugation methods, each offering distinct advantages in eliminating contaminants while maintaining high yields of the therapeutic product.

One of the most widely used approaches for removing adventitious agents is size- and density-based separation, primarily through ultracentrifugation and filtration. Density gradient ultracentrifugation, typically using cesium chloride or iodixanol gradients, has been a traditional method for purifying viral vectors such as AAVs and lentiviruses. This technique is highly effective in separating full and empty viral capsids, a critical factor in ensuring potency. However, scalability remains a challenge, making it less practical for large-scale CGT manufacturing. Tangential flow filtration provides a scalable and efficient alternative, utilizing membrane-based separation to remove host cell proteins, DNA, and viral contaminants while simultaneously concentrating the therapeutic product (Yoshimoto et al., 2021). Sterile filtration, utilizing 0.2- $\mu$ m pore-size filters, serves as a final viral safety step, capable of removing bacteria and some large viral contaminants. However, non-enveloped viruses and smaller adventitious agents may still evade filtration, necessitating additional clearance methods. Although ultracentrifugation offers high purity, scalability constraints can limit its feasibility for late-stage or commercial production. Chromatography-based methods, on the other hand, provide more scalable solutions but require extensive optimization to maximize yields while ensuring the removal of impurities and adventitious agents.

In addition to filtration, chromatographic purification methods are essential for removing process-related impurities and adventitious agents in CGT manufacturing. Ion-exchange chromatography (IEC) is commonly employed to eliminate residual host cell DNA and proteins by exploiting charge differences between viral particles and contaminants (Kaludov et al., 2002). Affinity chromatography has also emerged as a powerful tool, enabling the selective capture of viral particles through ligand-based resins that recognize specific capsid proteins (Brument et al., 2002). This method offers high specificity and scalability while improving downstream processing efficiency (Smith et al., 2008). Furthermore, size-exclusion chromatography is often employed as a final polishing step to separate viral particles from aggregates and impurities based on size, ensuring the highest level of purity before clinical application (Kaludov et al., 2002).

Given the importance of adventitious agent removal, regulatory agencies require extensive process validation to demonstrate effective viral clearance in CGT manufacturing. To meet global regulatory expectations, manufacturers conduct viral spiking and clearance studies at multiple purification steps, verifying reproducibility and the absence of adventitious agents. Additionally, NGS can also verify the

effectiveness of each purification step by confirming the absence of residual contaminants. By integrating multiple purification strategies with rigorous quality control, CGT manufacturers can enhance product safety while maintaining high yields of therapeutic viral vectors. The ability to effectively remove adventitious agents not only ensures compliance with global regulatory standards but also enhances the reliability and efficacy of CGT products for patients.

### Microbiologics as a partner in viral safety

As cell and gene therapies continue to progress toward more complex, personalized, and high-impact treatments, viral safety remains a foundational requirement for product quality and patient safety. The integration of advanced detection technologies — such as NGS — combined with well-characterized biomaterial controls and validated purification strategies, is essential to mitigate the risks posed by adventitious agents.

The future of viral safety in CGT manufacturing lies in a collaborative ecosystem that unites scientific innovation, regulatory alignment, and deep technical expertise. Microbiologics contributes to this evolving landscape through its specialized services and capabilities, including advanced NGS-based

adventitious agent detection and a state-of-the-art BSL-3 lab for handling high-risk organisms. Our BSL-3 facility allows for the development and validation of complex viral safety assays under stringent biosafety conditions, supporting both research and manufacturing requirements.

In addition, our synthetic virology platform enables the creation of de novo viral controls to support assay development and validation, addressing gaps in available reference materials for a broad range of virus families. By offering tailored biomaterial solutions and leveraging our expertise in next-generation sequencing, Microbiologics provides critical tools for comprehensive viral safety strategies.

As the CGT field moves toward global scalability and regulatory harmonization, the demand for rigorous, validated approaches to adventitious agent detection will only grow. Microbiologics is dedicated to enabling CGT innovators to meet these demands with confidence — partnering to ensure the safety, efficacy, and regulatory compliance of therapies that have the potential to transform patient outcomes worldwide.

For a full list of references and author bios, visit [cellgenetherapyreview.com](https://cellgenetherapyreview.com) 



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