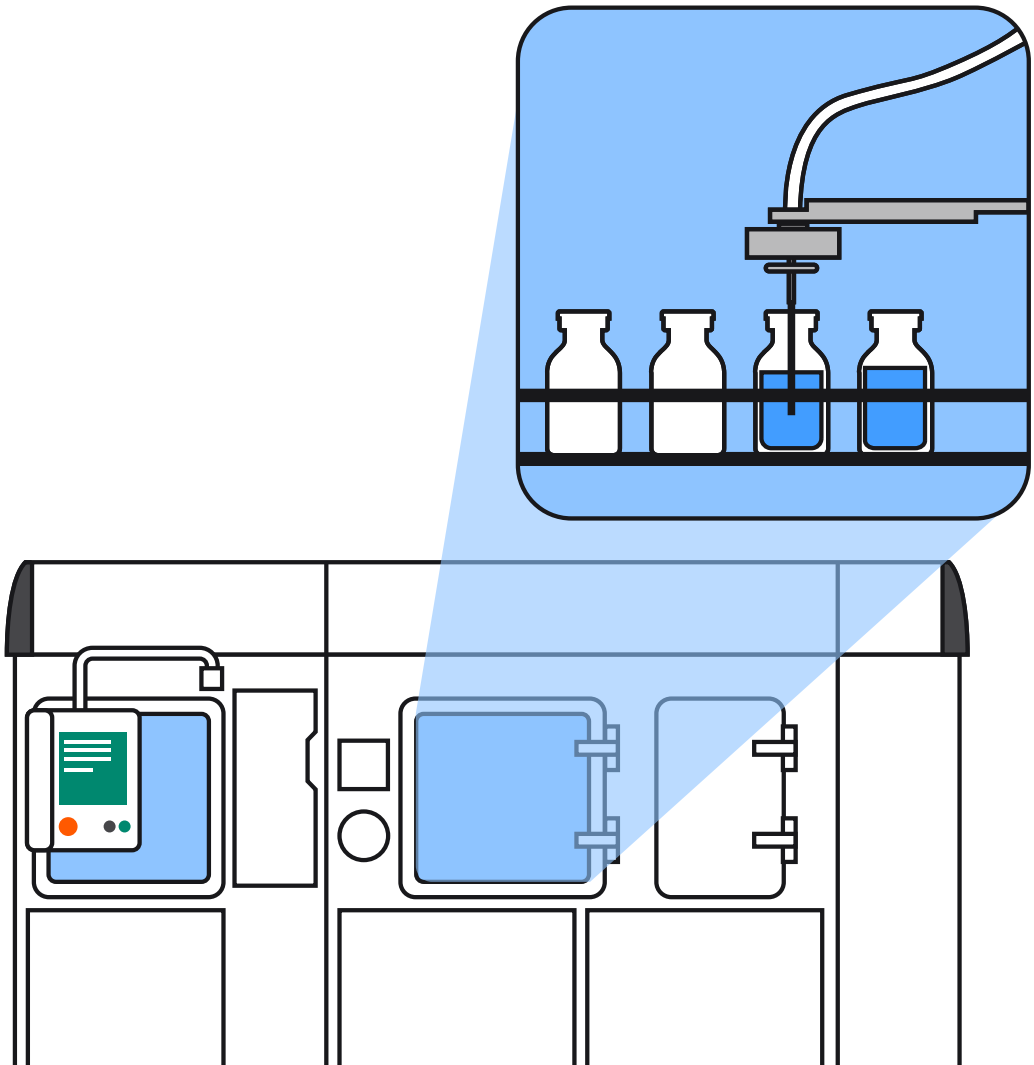
cytiva

Overcoming challenges in aseptic filling for ATMPs with gloveless isolators



cytiva.com

Executive summary

As the development of advanced therapeutic medicinal products (ATMPs) continues to grow, contamination remains a critical concern in ATMP manufacturing. Human intervention is the primary risk factor during the manufacturing process, necessitating advanced technologies to ensure sterility. Robotic gloveless isolators (RGIs) are an advanced technology solution to address this need, reducing contamination risks through automation, allowing for real-time continuous environmental monitoring, and minimizing human intervention to increase consistency in production quality. Additionally, RGIs offer the highest level of sterility assurance and fully comply with regulatory guidance as it evolves to meet the needs for aseptic filling of ATMPs.

Medicine in the 21st century is changing rapidly, and at least three major trends are driving this transformation. First, small molecule drugs, which dominated the therapeutic landscape for decades, are not suitable for some of the more challenging diseases that our society faces. Second, many uncommon or rare diseases are due to genetic mutations or immune dysfunction, and such conditions often require biological solutions. Third, a burst of scientific knowledge has opened the door to personalized medicine, which will eventually usher in an era of highly customized therapeutics (1, 2).

Combined, these factors have led to the advent of what the European Medicines Agency (EMA) calls Advanced Therapy Medicinal Products (ATMPs), which are therapies based on gene, cell, or tissue engineering. For example, Strimvelis, a gene-therapy product containing CD34+ cells engineered to express the human adenosine deaminase protein, was approved in the European Union (EU) in 2016 to treat the rare inherited condition known as severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID) (3). Another ATMP approved by the United States (US) Food and Drug Administration (FDA) in 2017, tisagenlecleucel, more commonly known under the brand-name Kymriah, is a CAR T cell therapy for the treatment of B-cell acute lymphoblastic leukemia (ALL) (4). Recently, 2023 saw the first approval in the EU and US of a CRISPR/Cas9 -mediated gene therapy, Casgevy (exagamglogene autotemcel), for the treatment of sickle cell disease (5).

While there are dozens more ATMPs approved for use in the EU and the US, they are often limited to use in the treatment of blood cancers or rare diseases. And, while 2025 promises to be a breakout year for ATMP approvals, the hope is that ATMPs will expand into more prevalent disorders and additional use cases, such as cardiovascular disease and solid tumor treatment for oncology (6).

The explosive growth of ATMPs

The FDA's approvals of the first three ATMPs in 2017—Kymriah, Luxturna, and Yescarta—initiated the promise of cell and gene therapies (CGTs), and in 2019 former FDA Commissioner Scott Gottlieb, M.D. and Peter Marks, M.D., Ph.D., Director of the Center for Biologics Evaluation and Research, issued a joint statement in which they predicted that the FDA would be on track to approve 10–20 new CGT products in 2025 (7). In 2024, nine new CGTs were approved by the FDA—including the first two adoptive cell therapies for treating solid tumors—nearly meeting that number one year earlier than Doctor Gottlieb and Doctor Mark's prediction (8, 9).

Looking beyond the first seven years of CGT approvals, the landscape for ATMPs is poised to expand quickly. According to a third quarter 2024 report from the American Society of Gene & Cell Therapy (ASGCT), a total of 32 gene therapies and 68 nongenetically modified cell therapies were approved and in use globally (10). Additionally, 4099 CGTs were in development at various stages from preclinical through preregistration. As ATMPs take their first steps addressing difficult challenges such as solid tumor treatment, they are poised for explosive growth in the coming years.

Therapies approved and in use globally:

32

Gene therapies

68

Nongenetically modified cell therapies

Source: Q3 2024 report from the American Society of Gene & Cell Therapy (ASGCT)

The demand for these drugs also has spurred regulatory agencies to create incentive programs (such as the FDA Breakthrough Therapy designation and European Medicines Agency's (EMA) PRIME designation) to expedite the development of therapies to manage serious chronic conditions with few, if any, treatment options.

These agencies also have issued updated guidelines and regulations that cover, among many other facets, guidelines for contamination control, sterility assurance, and aseptic filling for parenteral products. European Union Good Manufacturing Practice (EU GMP) Guidelines Annex 1: Manufacture of Sterile Medicinal Products (Annex 1) lays out the minimum requirements for contamination control and aseptic filling (11, 12). In turn, this has necessitated a higher level of process innovation, instrumentation, and technology to ensure first, that modern sterility assurance and aseptic filling processes will be able to meet the increasingly complex needs of new modality drugs as they are developed. And second, that manufacturing of ATMPs can remain uninterrupted and able to meet escalating demand.

To meet these needs, traditional aseptic filling protocols and equipment have fallen short, and new technologies have emerged to fill the gap and move the pharmaceutical manufacturing industry forward at scale. Chief among these are robotic gloveless isolators (RGI), which combine innovation in automation, robotics, ready-to-use (RTU) materials, and environmental monitoring to achieve nearly zero-count aseptic filling environments for completing the manufacture of the most advanced and delicate ATMPs on the market. Here we detail the current state of the aseptic filling industry, discuss where the gaps in technology exist concerning aseptic filling for ATMPs, and present the benefits of RGIs for closing those gaps and positioning the biomanufacturing industry to meet the aseptic filling needs of future ATMPs.

Contamination: Sources and consequences

Contamination can occur at any stage of the ATMP manufacturing process, and it is of particular concern during the final step, known as aseptic filling. Involving the transfer of a finished parenteral product from bioreactors to vials, syringes, or cartridges, the filling process typically represents the shortest segment of time that goes into the biomanufacturing of an ATMP. This final step is one of the most precarious moments in releasing a successfully manufactured therapeutic batch. Evidence of contamination in the filling environment, or even in just a single therapeutic unit, can spell doom for the entire manufacturing run.

Personnel remain the primary source of contamination, even as the technology surrounding aseptic filling improves. While inadequate training and protocol breaches can lead to contamination, the human body itself remains an inherent source of particulates, even under ideal conditions. Though estimates vary, humans shed millions of dead skin cells every day. Additionally, microbes—which colonize the entire surface of the skin—are carried along with these cells. And even in the absence of humans, the air itself is still impure.

A strict contamination control strategy involving cleanrooms, stringent filtration processes, and unidirectional positive-pressure airflow is critical to protecting drug product from viable and nonviable particulates throughout the biomanufacturing process. Typical indoor air might contain more than 35 million viable and nonviable particulates (≥ 0.5 microns in diameter) per cubic meter. To put that into perspective, the European Commission's Annex 1 guidance on cleanrooms defines the following standards for the various levels of cleanroom classification:

Table 1. European Commission's Annex 1 guidance on cleanrooms

Grade	Maximum limits for particulates ((≥ 0.5 microns/cubic meter)		Approx. reduction from average particulate concentrations	
	At rest	In operation	At rest	In operation
A	3520	3520	1000-fold	1000-fold
B	3520	352 000	1000-fold	100-fold
C	352 000	3 520 000	100-fold	10-fold
D	3 520 000	Not defined	10-fold	Not defined

Source: European Commission / Annex 1: Manufacture of Sterile Products (11)

If sterile products become contaminated, the consequences can be dire. At the very least, it will likely result in a product batch that must be discarded, costing the company time, resources, and wasted funding. Regulatory agencies like the FDA could issue warning letters, threatening the company with product recalls or legal action. For patients, the consequences can potentially be fatal.

Aseptic filling, therefore, is a process that has zero margin for error. How can this seemingly impossible task be accomplished as quickly and aseptically as possible?

Traditional aseptic filling methods

Aseptic filling has been performed using a wide range of methods and technologies. At the low-tech end are biosafety cabinets that allow an individual to access a work bench to manually fill containers. Biosafety cabinets often utilize HEPA filters and air flow to minimize contamination, but because the cabinet is open to the external environment, the risk of contamination remains high, even if housed inside a cleanroom. Additionally, biosafety cabinets necessitate manual filling, which introduces variability and is highly prone to human error, labor-intensive, time-consuming, and difficult to scale. Additionally, manual filling introduces moving obstacles into the laminar airflow, which can cause eddies and backflows within air currents that may potentially strip contaminating microbes and particles from personnel and introduce them into the filling environment.

Another filling method is the conventional filling line, which employs a series of machines that fill, stopper, and seal containers on a conveyor belt. In this way, automated systems can handle repetitive tasks at high speed and precision, which allows a product to be manufactured at scale. Though the entire system is housed within a cleanroom, the risk of contamination is still considerable, because the drug product is open to the environment. Potential contamination is also introduced as personnel enter and exit the cleanroom and risk interrupting airflow.

Restricted access barrier systems (RABS) are an improvement, as the system is at least partially enclosed from the external environment and requires personnel to interact with the drug product via glove ports. There are two types, open and closed, and as the names imply, closed RABS are more isolated than open RABS.

RGIs are structurally similar to closed RABS, but they are even more closed off from the environment and feature automated decontamination cycles. But even isolators can have contamination risks associated with isolator gloves, occluded surfaces, material transfer, and aseptic connections. In particular, conventional isolators typically lack several key components that would improve their utility for the aseptic filling of ATMPs: minimized filling loss, continuous real-time environmental monitoring of viable particles, and the elimination of glove ports, which would functionally remove manufacturing personnel from the aseptic filling task.

Robotic gloveless isolators and aseptic filling for ATMPs

Traditional methods of manufacturing biologics, including ATMPs, often involve highly manual processes, which can be prone to human error, time-consuming, and difficult to scale. With the advent of more advanced biomanufacturing automation technologies, manufacturers can achieve higher levels of productivity, accuracy, and efficiency. As increasing numbers of ATMPs are in development and these new modality drugs become more prevalent, one of the key challenges for their success is adapting the biomanufacturing and aseptic filling processes to meet the unique requirements of these therapies.

Since many ATMPs are biologically sensitive, containing living cells, viral vectors, or biologically active agents, maximizing sterility and minimizing contamination during production is crucial. To achieve this using traditional methods of aseptic filling, highly controlled environments and specialized cleanroom conditions are essential, along with the use of extremely strict aseptic techniques to prevent contamination. As such, manual filling processes become time-consuming, introduce dangerous variability, and increase the risk of contamination. As well, traditional pharmaceutical manufacturing involves high-pressure or high-flow filtration processes that can be destructive to the delicate nature of ATMPs. When products are cellular or viral in nature, filtration may not always be an option. These situations demand a minimization of product transfers between manufacturing and filling stations, which traditional filling cannot achieve.

These challenges have led to the evolution of specialized filling technologies like RGIs, which are adaptable to the complex needs of ATMPs. RGIs provide the most stringent possible parameters for aseptic filling.

The benefits of RGIs are substantial:

- **Robotic, automated systems** ensure a standardized process that produces consistent, repeatable results. The system is fully automated, capturing process parameters and system operations with any alerts or alarms communicated in real-time. Additionally, all movement of sterilized product contact surfaces avoids contact with decontaminated surfaces.
- **A gloveless barrier** ensures a completely isolated system with no operator inputs and minimal human involvement. Isolation is further enhanced by providing separate chambers for product input, filling, and stoppering, minimizing overall airflow disturbance.
- **Positive pressure unidirectional airflow** moves from critical to noncritical areas, minimizing turbulence to reverse airflow.
- **Internal decontamination cycle** using vaporized hydrogen peroxide (VHP) ensures decontamination of nonproduct contact surfaces in the isolator while minimizing residual VHP.
- **Real-time continuous environmental monitoring** simultaneously monitors viable and nonviable particles, ensuring real-time detection of contamination and minimizing batch loss.
- **Built-in scalability and adaptability** ensure flexible production capacity to accommodate multiple products and nested container formats.

RGIs allow for fully aseptic transfer from material input to container filling through stoppering, eliminating several nodes of potential contamination. By automating this process, robotic systems can handle repetitive tasks with high speed and precision, ensuring the correct fill volumes and minimizing the risk of contamination, in many cases creating consistently zero-count filling/stoppering environments. The increasing complexity of ATMP formulations has prompted the development of innovative filling systems and single-use technologies that maximize sterility and minimize cross-contamination.

As demand for ATMPs grows, automation is increasingly becoming integral to scaling up production. Automation tools, such as RGIs and real-time monitoring technologies like biofluorescent particle counters (BFPCs), are enhancing the efficiency and consistency of the filling process. BFPCs can instantly detect contaminants or particles in the air, offering real-time data that can be used to adjust operations immediately if necessary. Such technology enhances the precision of the production process, contributing to the final product's safety and quality.

Many ATMPs, especially in the gene and cell therapy sectors, are tailored to individual patients, necessitating personalized, patient-specific manufacturing. This presents challenges in manufacturing and filling, requiring systems capable of handling smaller, variable batch sizes. RGIs accommodate fluctuations in batch sizes and manufacturing volume, allowing manufacturers to meet patient-specific demands without compromising on regulatory compliance or quality standards.

Meeting new regulatory requirements

Regulatory agencies are beginning to adopt and implement stricter requirements for aseptic filling, particularly for environmental monitoring. For decades, the standard technique for environmental monitoring of viable particulates was—and in many cases, still is—placing dozens of plates filled with growth-promoting media agar throughout the aseptic filling environment in an attempt to catch microbial particles as they settle out of the atmosphere around the filling environment and the drug product. This passive, low-tech method for detecting contamination has several drawbacks: (1) Plates filled with growth-promoting media agar must be changed frequently, which introduces the risk of external contamination; (2) These plates must be incubated to see microbial outgrowth, which typically takes several weeks, creating delays in the release of manufacturing batches; (3) Not all microbes are cultivatable on plates filled with growth-promoting media agar; (4) Plates filled with growth-promoting media agar often don't catch many particles at all especially if in unidirectional airflow – often capture rate is below 10%; (5) Nonviable (abiotic) particles, such as fungal and bacterial spores, flakes of skin, or fibers, cannot be detected at all.

These limitations create substantial risk for a manufacturer. Batches of product cannot be shipped until it is determined that no contamination was present in the isolator. But this can take up to two weeks, as slow-growing microbes need time to incubate. Worse, if contamination is eventually found, the entire batch likely must be discarded – a waste of time and resources.

For reasons such as this, the EU has issued updated regulations in "Annex 1: Manufacture of Sterile Products" that emphasize continuous monitoring of nonviable (§ 9.17) and viable (§ 9.27) particles, highlighting the importance of real-time data to ensure proper contamination control within aseptic manufacturing environments (11).

RGIs like the SA25 aseptic filling workcell approach real-time environmental monitoring with newer technologies like biofluorescent particle counters (BFPCs). Using real-time laser-mediated interferometry, BFPCs can detect both nonviable (abiotic) and viable (biotic) particulate matter as it is encountered in real-time within the positive pressure airflow in an aseptic filling chamber. For ATMPs, steady, clean, unidirectional airflow that sweeps over and away from the aseptic filling area is critical, and as such, BFPCs are an ideal technology for monitoring the filling environment for viable and nonviable particulate contamination.

In addition to being accepted by the FDA for aseptic manufacturing and referenced by EMA in Annex 1, this laser-based system confers multiple benefits:

- ☑ **Real-time sensitivity** allows for early warning and timely interventions.
- ☑ **High degree of accuracy** lowers false positive and false negative rates.
- ☑ **Ability to distinguish different types of particles** enables enhanced quality control.

Continuous, real-time monitoring means that, in the event of a warning, the manufacturing process can be halted immediately. Thus, instead of losing an entire batch of product, only a single tray may need to be discarded. Crucially, a BFPC also eliminates the waiting period necessitated by growth-based environmental monitoring methods.

The combination of completely automated, robotically mediated filling technology, continuous positive-pressure airflow, and BFPC-based real-time continuous environmental monitoring that RGIs such as the SA25 aseptic filling workcell bring together can meet the most stringent regulatory requirements for aseptic filling. This configuration is also well positioned to meet the evolving regulatory demands that will inevitably emerge as therapeutic complexity increases in the future.

Conclusion: The case for robotic gloveless isolators

As 21st century medicine continues its march forward, the need for robotic gloveless isolators (RGIs) will become increasingly apparent. Patient safety is paramount, and RGIs can consistently ensure the delivery of high-quality, high-purity products. Beyond patient safety and regulatory compliance, there are sound business reasons to invest in an RGI. Particularly for ATMPs, batch sizes are smaller and more variable, and RGIs are constructed to be adaptable to the changing needs of small- and medium-sized batches.

Finally, the high cost of ATMPs presents a significant barrier to their widespread adoption. Developing more cost-effective manufacturing and filling methods will be critical to making these innovative therapies more affordable. By ensuring quality and decreasing the time to market, RGIs can lower the cost of production and can potentially benefit patients and healthcare systems alike.

Learn more today.

References

1. Johnson KB, Wei W, Weeraratne D, et al. Precision Medicine, AI, and the Future of Personalized Health Care. Clin Transl Sci. 2020;14(1). doi.org/10.1111/cts.12884.
2. Cinti C, Maria GT, Joulie M, Ayoub H, Frenzel M. The Roadmap toward Personalized Medicine: Challenges and Opportunities. *J Pers Med.* 2024;14(6):546-546. doi.org/10.3390/jpm14060546.
3. European Medicines Agency. Strimvelis - European Medicines Agency. Published September 17, 2018. https://www.ema.europa.eu/en/medicines/human/EPAR/strimvelis Accessed February 3, 2025.
4. Awasthi R, Maier HJ, Zhang J, Lim S. Kymriah (tisagenlecleucel) – An overview of the clinical development journey of the first approved CAR-T therapy. *Hum Vaccin Immunother*. 2023;19(1). doi.org/10.1080/21645515.2023.2210046.
5. Vertex and CRISPR Therapeutics Announce Authorization of the First CRISPR/Cas9 Gene-Edited Therapy, CASGEVY (exagamglogene autotemcel), by the United Kingdom MHRA for the Treatment of Sickle Cell Disease and Transfusion-Dependent Beta Thalassemia | Vertex Pharmaceuticals. Vertex Pharmaceuticals. Published 2023. https://investors.vrtx.com/news-releases/news-release-details/vertex-and-crispr-therapeutics-announce-authorization-first Accessed February 3, 2025.
6. Saionz A. Cell & Gene Therapies in 2025: Breakthroughs, Challenges, and the Path to Accessible Innovation. *PharmaBoardroom.* Published January 7, 2025. https://pharmaboardroom.com/articles/cell-gene-therapies-in-2025-breakthroughs-challenges-and-the-path-to-accessible-innovation/ Accessed February 3, 2025.
7. Scott G, Peter M. Statement from FDA Commissioner Scott Gottlieb, M.D. and Peter Marks, M.D., Ph.D., Director of the Center for Biologics Evaluation and Research on new policies to advance development of safe and effective cell and gene therapies. FDA. Published March 24, 2020. https://www.fda.gov/news-events/press-announcements/statement-fda-commissioner-scott-gottlieb-md-and-peter-marks-md-phd-director-center-biologics Accessed February 3, 2025.
8. Pategou J. Catalysts of Change: How the Cell and Gene Therapy Market Has Evolved. Cell & Gene. Published 2025. https://www.cellandgene.com/doc/catalysts-of-change-how-the-cell-and-gene-therapy-market-has-evolved-0001 Accessed February 3, 2025.
9. Kishton, RJ, Restifo, NP. T cells lead the charge against solid tumors. *Nat Cancer.* 2024; 5(12):1762-1764. doi.org/10.1038/s43018-024-00860-8.
10. American Society of Gene & Cell Therapy. Gene, Cell, & RNA Therapy Landscape Report Q3 2024 Quarterly Data Report. https://www.asgct.org/global/documents/asgct-citeline-q3-2024-report.aspx Accessed February 3, 2025.
11. European Commission. Annex 1: Manufacture of Sterile Products. https://health.ec.europa.eu/system/files/2020-02/2020_annex1ps_sterile_medicinal_products_en_0.pdf Accessed February 3, 2025.
12. European Commission. The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use. https://www.gmp-compliance.org/files/guidemgr/20220825_gmp-an1_en_0.pdf Accessed February 3, 2025.

cytiva

cytiva.com

Cytiva and the Drop logo are trademarks of Life Sciences IP Holdings Corporation or an affiliate doing business as Cytiva. Any third party trademarks are the property of their respective owners. The Danaher trademark is a proprietary mark of Danaher Corporation.

© 2025 Cytiva

For local office contact information, visit cytiva.com/contact

CY50839-21Feb25-WH

danaher