Simplifying Progress

Optimized Aliquoting for Robust Cell and Gene Therapy Processes

April, 2023 | Maria Rende, Prisca Baptista, Katy McLaughlin

Keywords or Phrases:
Aliquoting, cell and gene therapy

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Introduction

Aliquoting is ubiquitous across bioprocessing operations as media and buffers are often formulated in bulk and must be aliquoted into smaller containers for simpler transport in the facility and easier handling at the point of use. For example, cell culture media are typically prepared and distributed into dozens or hundreds of smaller containers (such as single-use bags) for use in operations. While aliquoting seems, conceptually, a simple operation, it has a significant impact on process efficiency and robustness and therefore warrants consideration as part of continuous process improvements.

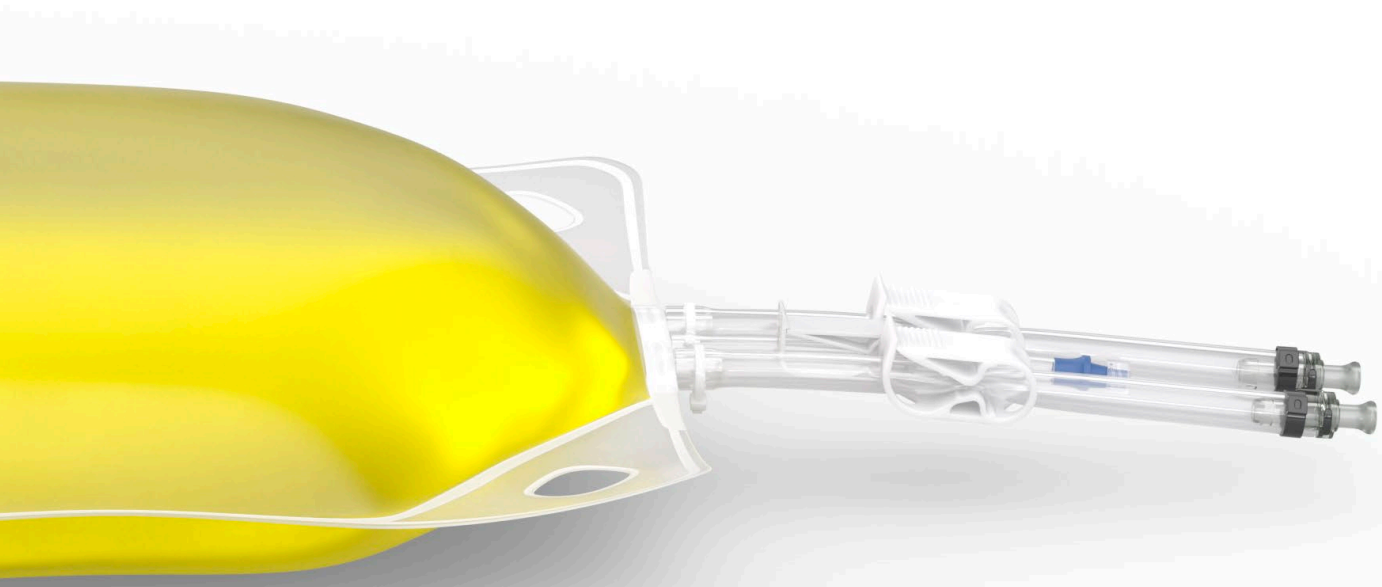
Optimized aliquoting is particularly important in advanced therapies, such as cell and gene therapies, which are typically high-value, low-volume applications that require careful handling of small volumes. Advanced therapies also lack the platform approaches that are commonplace in more traditional biotherapeutics, such as monoclonal antibodies (mAbs). This is partly due to their inherent variability (between patient samples, for example) but also because the field is still relatively novel, with techniques and regulatory standards continuing to evolve.

In this white paper, we consider the challenges associated with liquid handling in cell and gene therapy processes and introduce some novel solutions that simplify aliquoting in modern bioprocesses.

Aliquoting — an Often-Overlooked Pain Point

Manual filling carried out under aseptic conditions requires extensive material and equipment, preparation, environmental monitoring, complex repetitive tasks, multiple operators, and hours of employee time. Manifold filling can reduce manual intervention during the manipulation of large volumes. However, it still requires attentive focus on the volume dispensed. As a result, these processes are highly resource-intensive, operationally-challenging, error-prone, and costly.

On the other hand, automated filling systems minimize variability and workload. However, they typically involve large, complex equipment, requiring significant upfront investment to install and maintain. They are also not suitable for multi-product facilities where a machine may need to fill different volumes and therefore require multiple calibrations or multi-site manufacturing networks. Current trends in the biopharmaceutical industry such as the growing interest in cell and gene therapies and decentralized manufacturing have increased the need for lower-volume applications. To keep up with these process requirements, bioprocessing scientists require cost-effective dispensing solutions for the accurate handling of small volumes that help streamline unit operations.



Aliquoting Advanced Therapeutics

Traditional biotherapeutics, like recombinant proteins, mAbs, and vaccines, have well-established manufacturing processes typically built with the aim of producing large product volumes for large sets of the population at a clinical or commercial scale. New modalities – like many cell and gene therapies – lack standardized processes to overcome bioprocess challenges, such as their comparably much smaller batches, more limited distribution (either one or few individuals), and extremely custom, high-value products. Furthermore, individualized therapies involve the simultaneous handling of multiple samples for many different patients. Processes must be designed and equipment selected to control costs and meet the demands of single-patient therapies without risking the reliability of the entire manufacturing process. Aliquoting demand might differ depending on individual processes and therapy types (autologous or allogeneic).

In allogeneic therapies, cells are sourced from a healthy donor cell bank and are expanded to produce larger batches (albeit still significantly smaller than traditional biotherapeutics) that can be divided into many doses and given to multiple patients. Therefore, the amount of media required can be easily fixed (or standardized) and produced in large volumes . However, media must be repeatedly added to culture vessels during cell expansion, which is challenging with large-volume containers; and using smaller (i.e., 1 L) bags is typically more convenient (Figure 1).

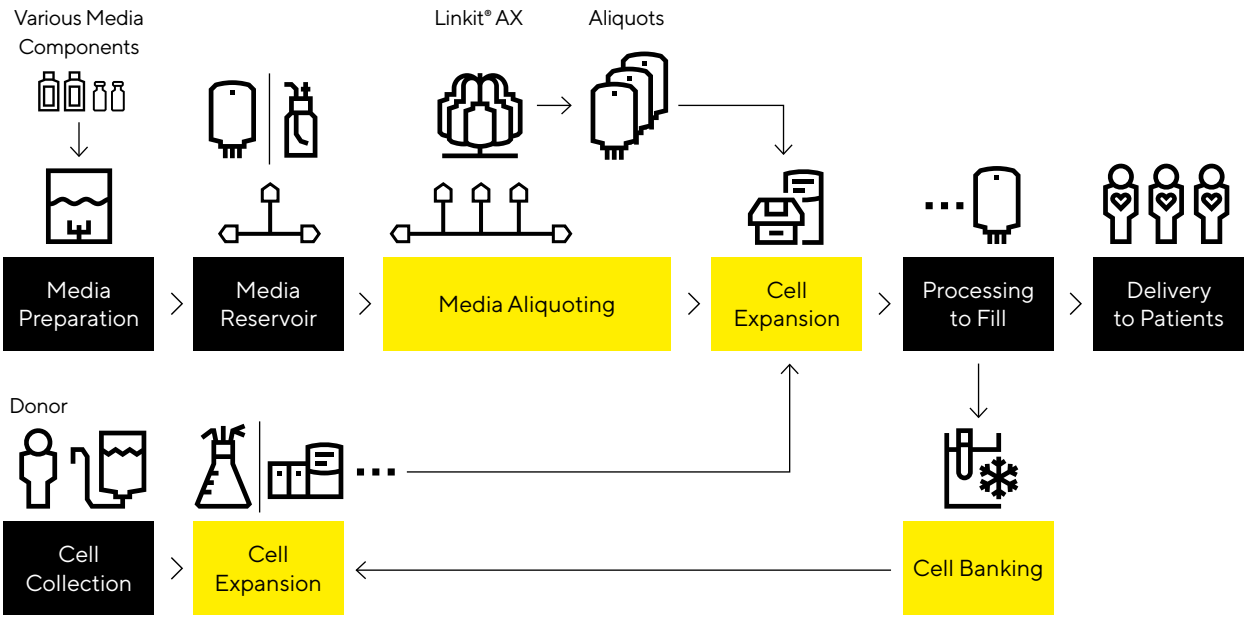
In contrast, autologous therapies involve collecting cells from the patient, engineering them, and returning them to the same patient (Figure 1). This places a significant demand on operators. For example, during a chimeric antigen receptor (CAR-T) cell therapy production process, patient cells are cultured in a bioreactor which is fed by multiple small media bags added at different stages of growth. The media can be prepared in bulk but must be distributed into 100 – 1,000 mL bags to allow its delivery in small batches. A single media batch may be 50 – 500 L and produced every 2-3 days, requiring the filling of hundreds or thousands of bags per week – an operationally-challenging and resource-intensive task. This can increase the chance of errors, which are hugely problematic in the production of these types of therapies, as a patient's life could depend on receiving the personalized treatment.

An additional challenge associated with aliquoting in autologous therapies is the variability associated with using the patient's own cells as starting material and, consequently, the volume of media required to culture and harvest them. Aliquoting at smaller volumes reduces the potential of media loss from partially consumed media bags since any excess media from a large batch cannot be used for other patients. This limits the waste of large quantities of unused media, which is highly expensive.

These unique process challenges highlight the inefficiencies bioprocess scientists face when performing "simple" process steps. Currently, drug developers are spending valuable time searching for multiple individual solutions to integrate and build an efficient and streamlined process.

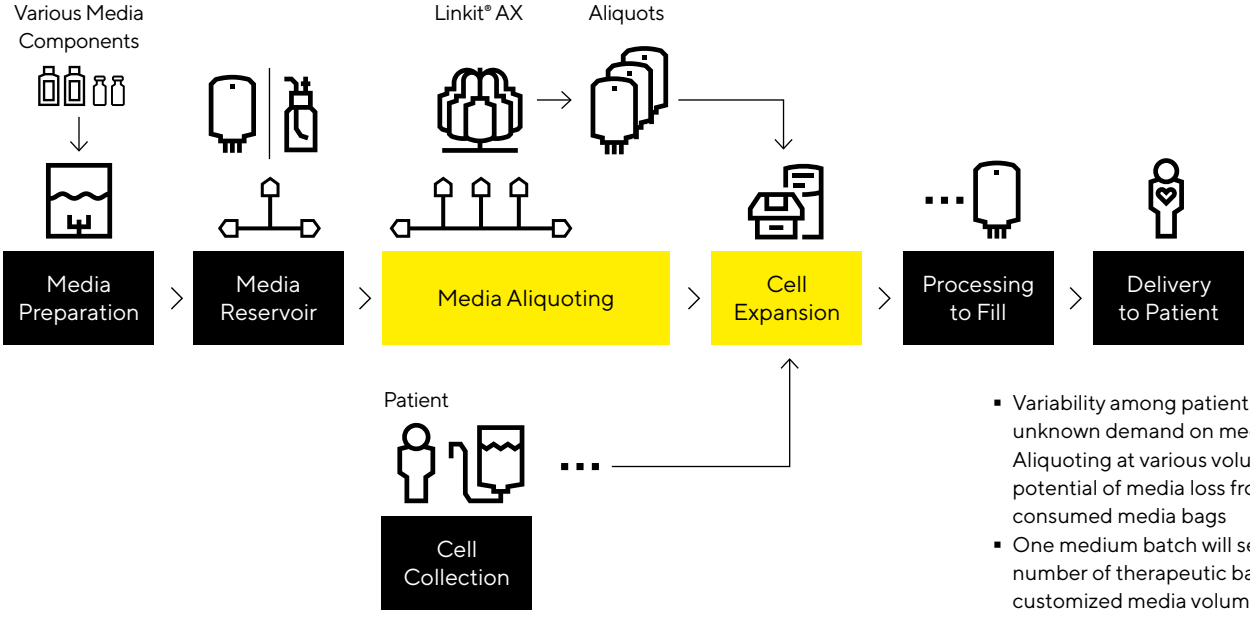
Figure 1: *Autologous and Allogeneic Therapy Process Workflows*

Allogeneic Therapies



- Aliquoting media at various volumes supports simplified handling when cells are expanded from small to large culture vessels
- Media aliquots can be prepared in advance to initiate cell culture process from cryopreserved cells

Autologous Therapies



- Variability among patient cells means unknown demand on media consumption. Aliquoting at various volumes reduces potential of media loss from partially consumed media bags
- One medium batch will serve a smaller number of therapeutic batches for customized media volume preparation

How is the Industry Adopting Smarter Strategies?

A continuous, automated, closed system would be optimal to avoid contamination and reduce labor-intensive multi-step processes while limiting potential human error.

As well as reducing employee hours, speed overall is a huge business driver. A system that operates quickly would help speed up process development and improve manufacturing efficiency, allowing reductions in time-per-batch and speeding up time to market.

An aliquoting solution with traceability features would help provide control over this procedure. This is essential to demonstrate consistency and accuracy, which are vital for compliance and patient safety. Additionally, traceability also helps production control as it supports supply chain management.

Finally, keeping costs low is always an important driver. Ideally, manufacturers will want to limit investment into expensive, bulky equipment to keep the process lean and more economical overall. Cost reductions might be measured by reductions in timelines, employee hours, errors, and batch losses.[1]

Linkit® AX – an Optimal Integration Tool

The Linkit® AX represents an improved solution for liquid handling between process steps in a continuous workflow. It is an ideal tool for aliquoting small quantities of fluid in applications such as small-batch media preparation in cell and gene therapies.

Figure 2: *Overview of the Linkit® AX Aliquoting Solution.*

Linkit® AX Single-Use Assembly:
Enables aliquoting into 10 Flexsafe® bags simultaneously.

QApp Linkit® AX:
A software package developed to optimize filling performance during aliquoting applications. Set-up once, aliquoting only requires a few simple steps thereafter.

Pump:
A peristaltic pump connected and controlled by the balance.

Purge Stand:
A support to correctly position the air purge solution and the inlet tubing of the Linkit® AX consumable.

Cubis® II Balance:
A modern balance with a large and intuitive user interface, connectivity options for data extraction, 21 CFR part 11 compliant.

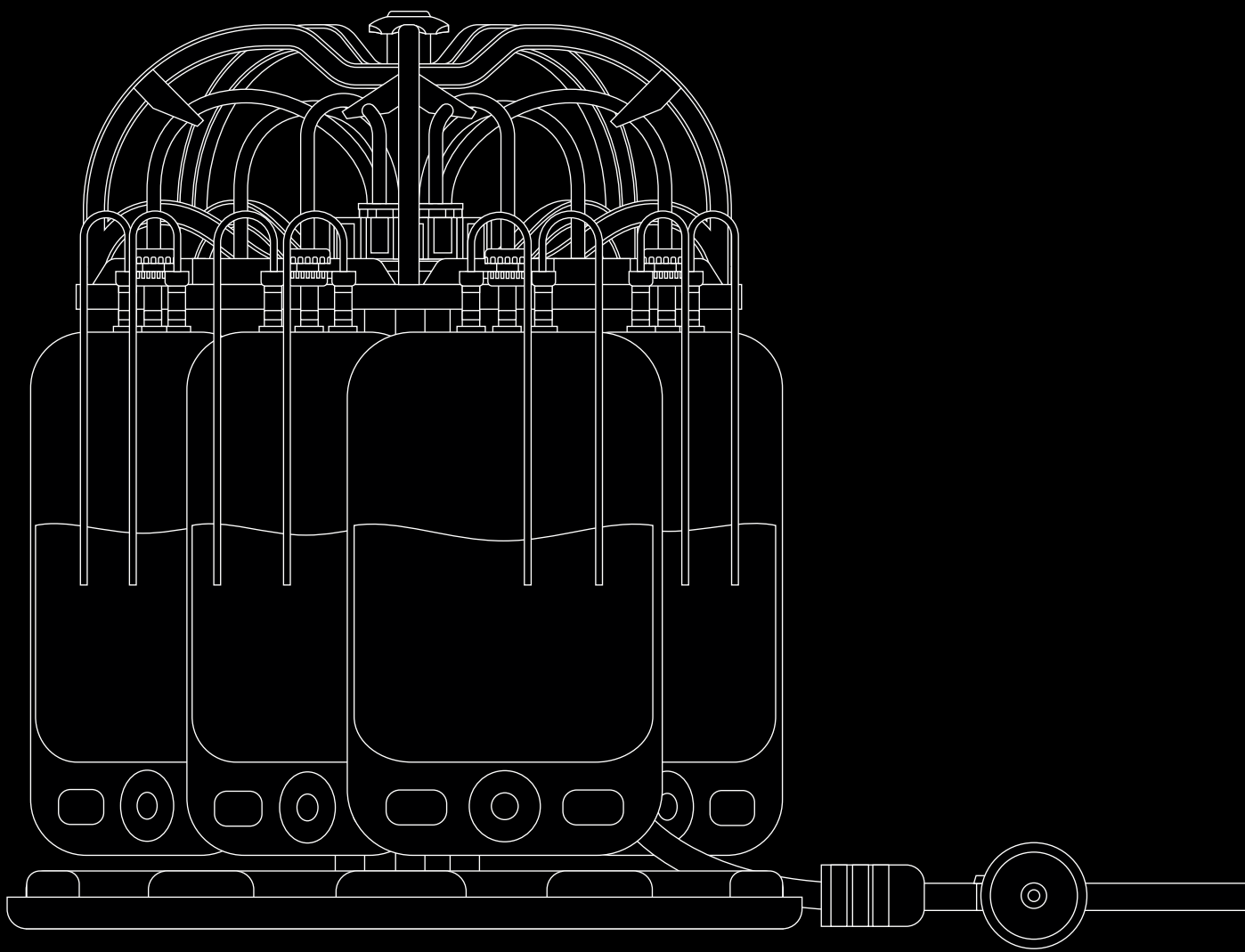
Benefits of the Linkit® AX

Workflow Integration & Optimization
The Linkit® AX Aliquoting Solution is easy to integrate into the larger workflow, creating a simplified and streamlined process. It comes fully assembled and is compact, unlike other bulky automated aliquoting systems.

Data Compliance
The system enables end-to-end traceability in compliance with FDA directive 21 CFR part 11 and EU Annex 11.[2] This easy record-keeping improves product control and patient safety.

Speed & Efficiency
The system is plug-and-play, limiting the expertise and training required for performance and maintenance. It also enables a streamlined process design, removing steps and speeding up parts of the process.

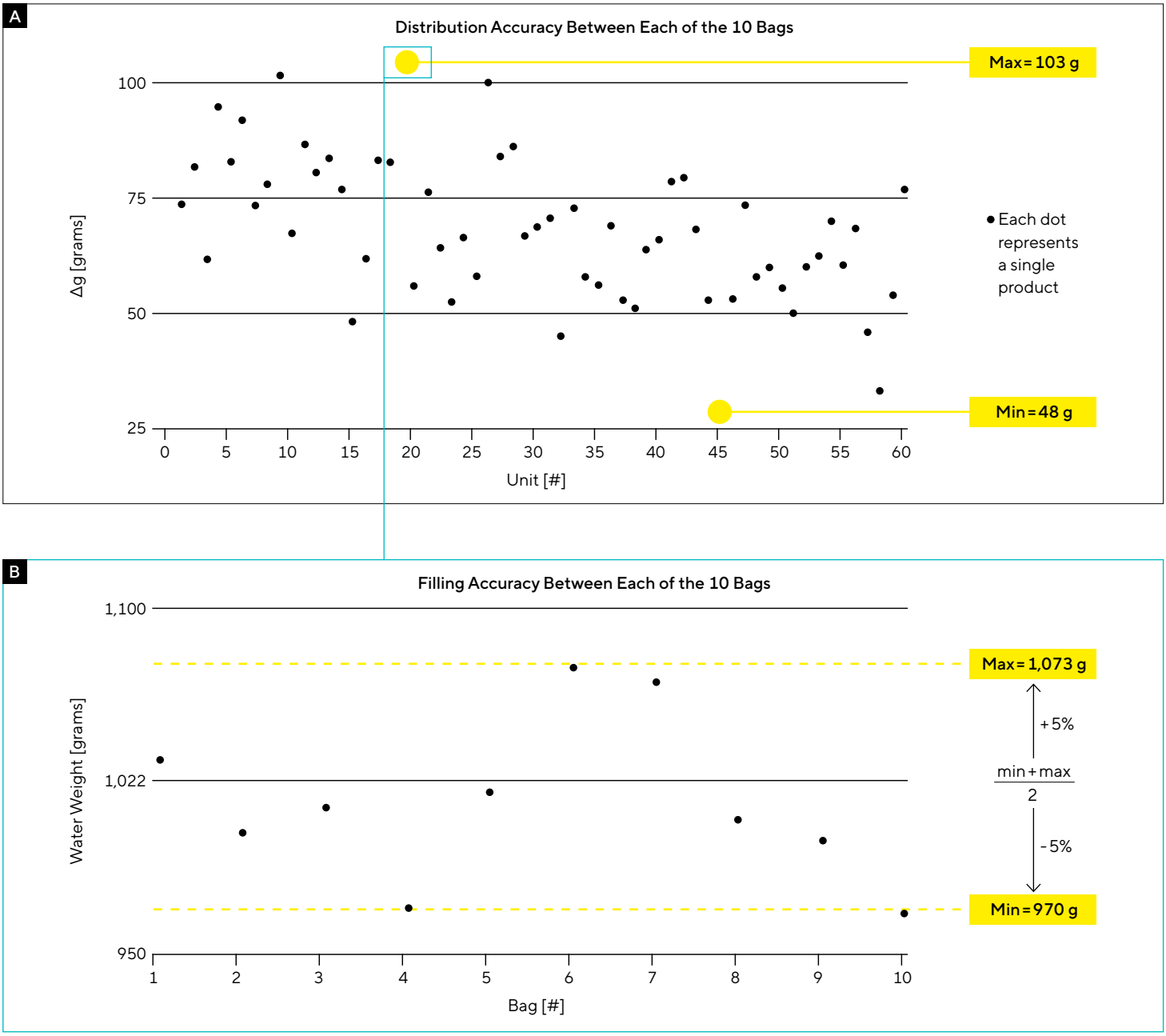
Automation
Linkit® AX limits manual interaction, saving operator time and eliminating a source of potential variation and error.



A Closer Look: Linkit AX® Filling Accuracy

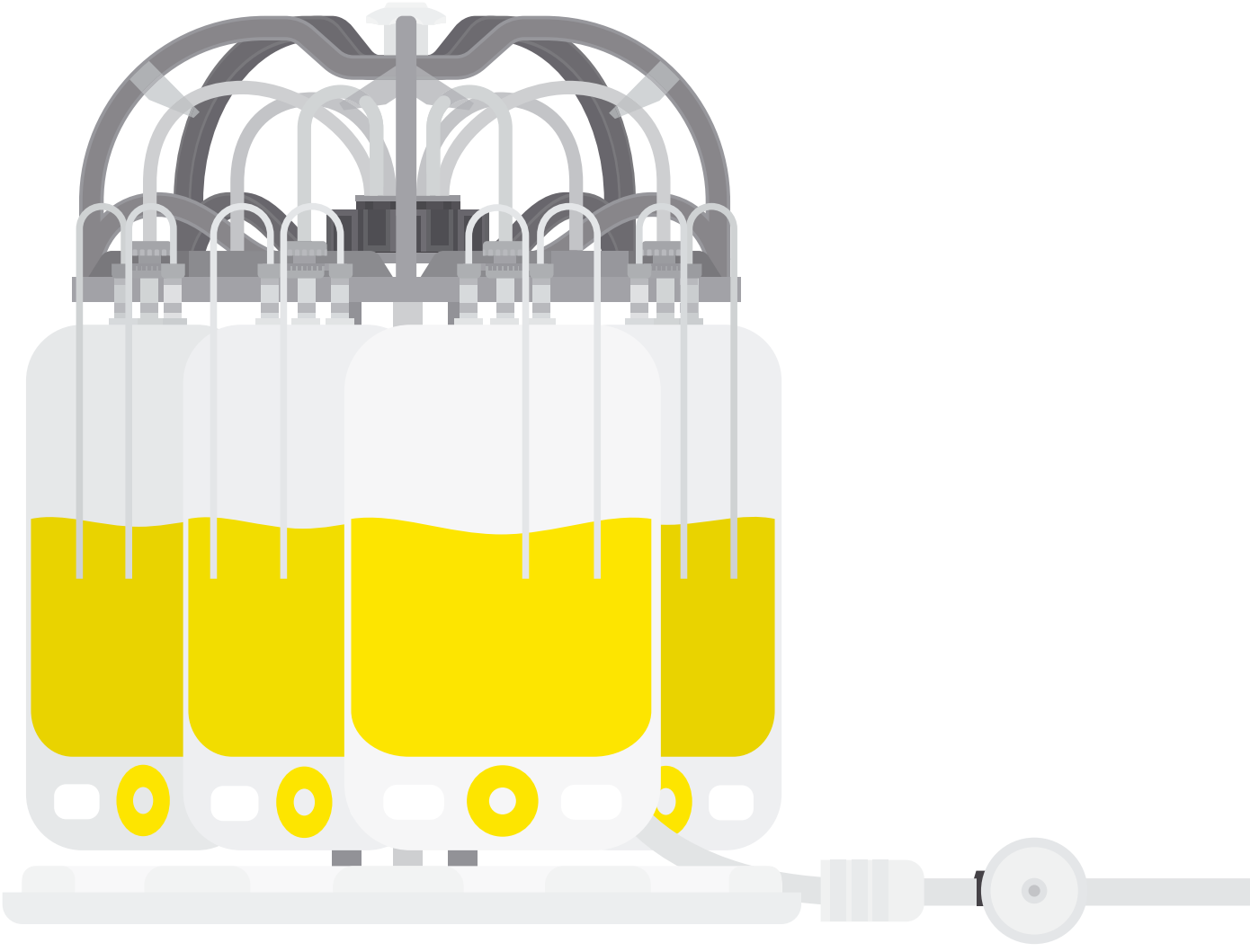
The Linkit® AX helps to minimize filling time by filling ten bags simultaneously, quickly, and evenly, achieving a distribution accuracy of ± 5.1 – 6.5%[3] between bags (Figure 3). As such, scientists do not have to invest in expensive equipment or compromise on speed to achieve accurate filling.

Figure 3: *Filling Accuracy of the Linkit® AX.*



Note. (A) Distribution results of the tested 60 units of Linkit® AX configuration FYM312731 (1,000 mL). Once the total target weight was reached, each of the 10 bags was removed and weighed to determine the accuracy of distribution between the bags. The Δ in grams between the heaviest and lightest bag was recorded.
(B) A deep dive into the worst-case unit (Δg = 103 between heaviest and lightest bag) shows a distribution accuracy of ± 5 % vs the average between the min and the max.

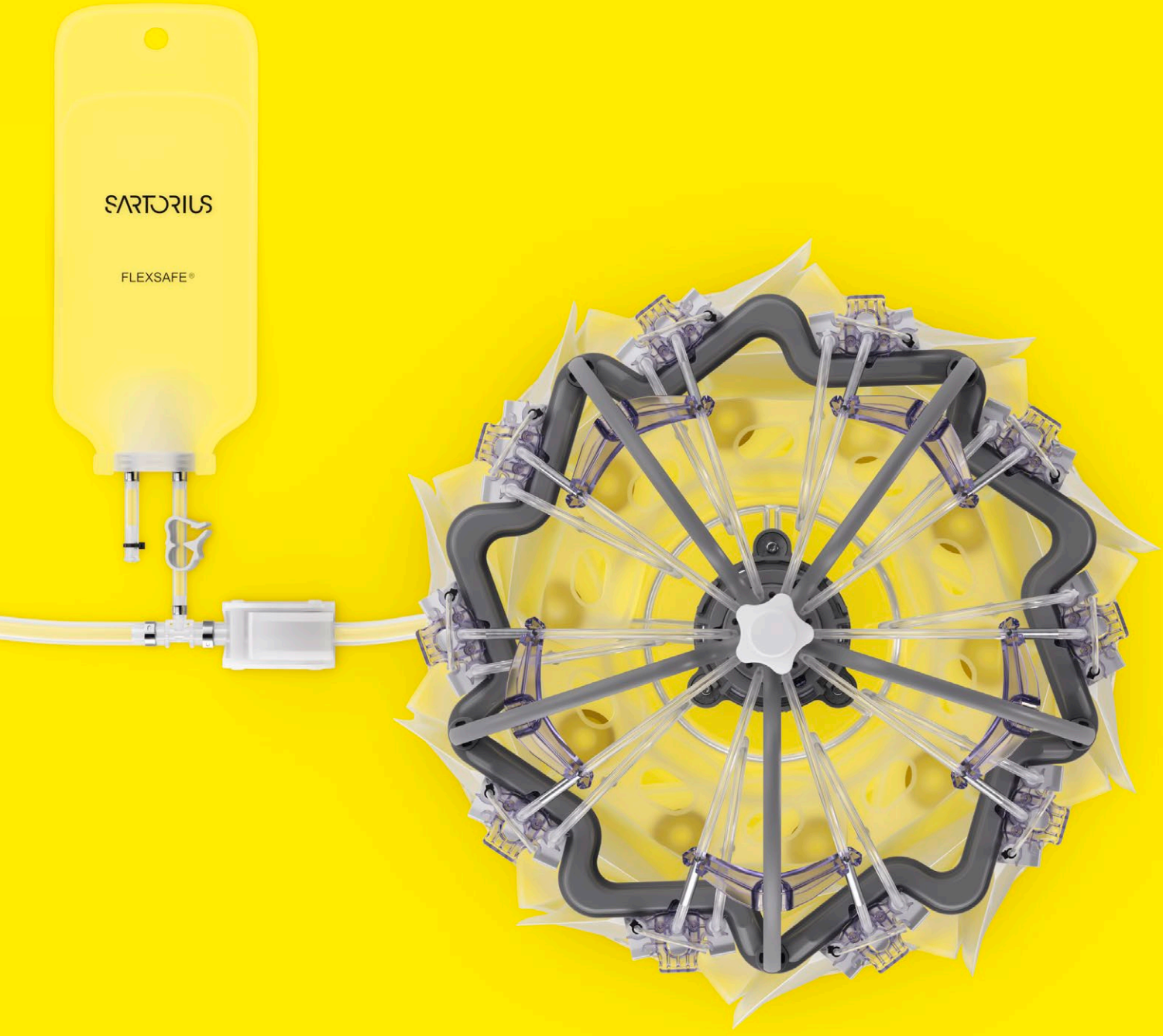
With these features, the Linkit® AX supports key business drivers. The platform facilitates the conservation of media to make a more sustainable and streamlined process, reducing costs and time-to-market. It also enables biopharmaceutical developers to respond to changing market trends, such as the need for new technologies to support the manufacture of precision medicines, which typically require smaller batch sizes, and the growth in decentralized manufacturing.



Conclusion

Efficient liquid handling is a vital element of any robust bioprocess. The Linkit® AX Aliquoting Solution is ideal for quickly, reproducibly, traceably, and sustainably dispensing small quantities of process fluids. The solution is designed to address the pain points of manual filling and aliquoting without the need to invest in large, expensive equipment.

Gain efficiency in your day-to-day liquid handling with minimal investment. Linkit® AX is your smart choice for bioprocess aliquoting.



Author Bio

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Maria is leading the Cell Therapy subject-matter within the Fluid Management Technology Marketing team and is responsible to provide a deep understanding of cell therapy processes, user requirements and market insights.

After completing her PhD in Biochemical Engineering, she continued to build upon her extensive knowledge in Cell Therapy process development and manufacturing throughout her 6 years tenure as research associate at Imperial College London and afterwards various business development positions, the last of which brought her into Sartorius in 2018.

Prisca Baptista
Product Manager
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Prisca Baptista has been working for Sartorius since 2017, where she is Product Manager for Transfer and Distribution Portfolio. She earned her engineering degree at the "Engineering European School for Material Sciences" in Nancy, France. Prisca started her career as project manager in the field of development for fluid management technologies. She has been working as well in Germany on the development of purification technologies.

As of 2021, she has started as product manager for tubes, tubing assemblies and worked on the launch of Linkit® AX solution.

Katy McLaughlin
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Katy is part of the Marketing Communications team at Sartorius, where she supports the creation of a variety of written pieces, from published articles to web content.

Before joining Sartorius in 2021, Katy was employed as a Post-Doctoral Research Associate at the University of Edinburgh, where she also completed her doctoral studies. Here, she carried out research in genetics and cellular biology and began taking on writing projects, eventually entering into a
career as a freelance writer for various biotech companies and agencies.

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3. ± 5.1% for 250, 500 and 1,000 mL bags | ± 6.5% for 150 mL bags

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Status: 09 | 2023