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Application Note

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Ambr® 250 Modular
T Cell Expansion for Cellular Immunotherapy

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Introduction

With the commercialization of promising Chimeric Antigen Receptor T cell (CAR-T) therapies, cellular immunotherapy has gained significant momentum in the field of cell and gene therapies (CGT). In this application note, we demonstrate the expansion of CAR-T cells in the Ambr® 250 Modular system.

Currently, cell culture flasks for adherent cultures and spinner flasks for suspension/suspension-adapted processes are widely used in process development and rocking motion bioreactors in manufacturing. These cell expansion platforms are labour-intensive, require a high level of highly skilled operator manipulation and often offer low level of culture monitoring and control. Moreover, cells for CGT applications are often scarce and many reagents have a high cost.

The Ambr® 250 Modular system provides a cost-effective and rapid way of evaluating critical process parameters (CPPs) for these cutting edge bioprocesses, whilst maintaining critical quality attributes (CQAs) to ensure safe

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and robust cell culture expansion. It also allows to introduce monitoring and control of CPPs in an automated way to better understand the process and aid the much needed process development in the field of CGTs.

With assistance from Sartorius Stedim Biotech, a very well recognized Cellular Therapy Company evaluated the Ambr® 250 Modular for the successful expansion of T cells.

Ambr® 250 Modular

Proof of concept for T cell expansion

The main aim of the evaluation was to transfer a T-cell process from a spinner flask (standard process i.e. control) into the Ambr® 250 Modular. Moreover, an evaluation of which vessel (baffled vs unbaffled) would be more suitable, was performed. Process analytical tools (PAT) were used to gather online, real time data on critical process parameters which were recorded and viewed using the Ambr® 250 Results Viewer software.

The secondary aim was to demonstrate that vessel design (standard with baffles versus unbaffled) can be critical to growth, proliferation and other Critical Quality Attributes (CQAs) such as phenotype in the case of T cells.

This study includes a side by side comparison of a spinner flask 'control' a standard vessel with baffles and the unbaffled single impeller vessel to assess the relative performance of the three vessels as measured by cell growth and detailed CQAs. The two different Ambr® 250 Modular vessels were run in parallel in a two bioreactor position Ambr® 250 Modular system.

An introduction to automated processing

The Ambr® 250 Modular is a benchtop bioreactor system for parallel cell culture in single-use vessels with 100-250 mL working volumes. The system comprises a series of benchtop modules enabling up to 8 bioreactors to be operated in parallel and a control module with intuitive system software accessed via a user-interface screen capable of automating feed additions.

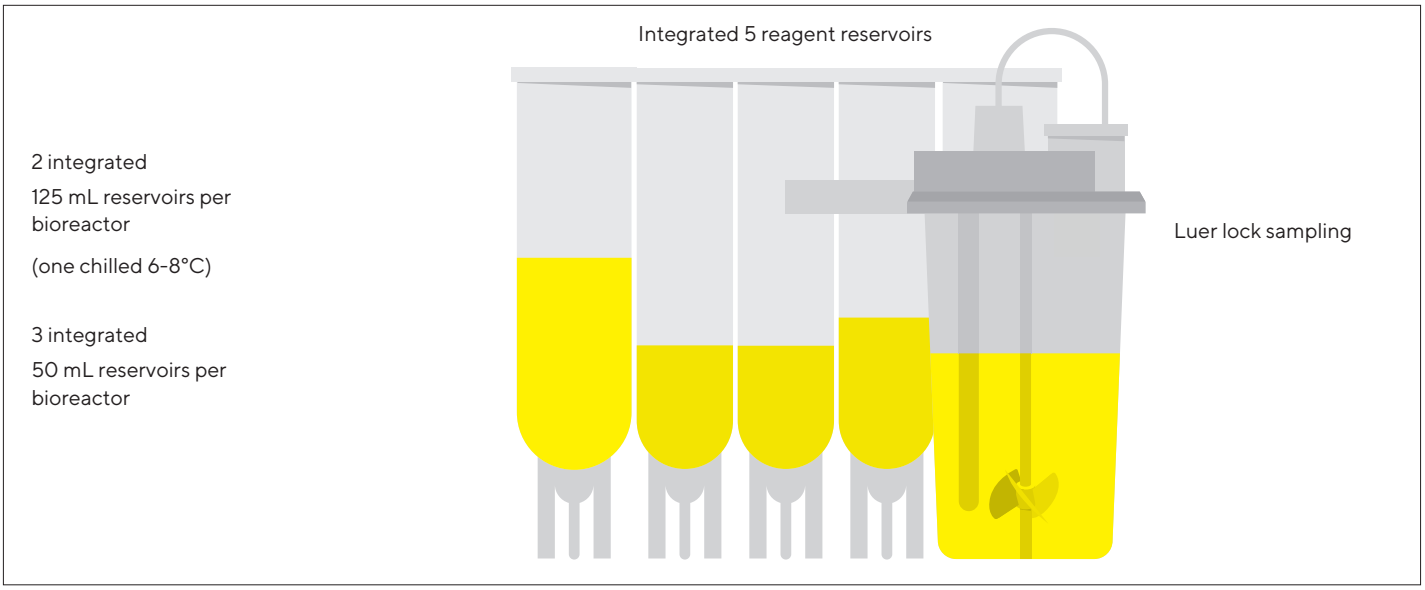
Each single-use bioreactor vessel (Figure 1) is fully integrated with sensors (temperature, pH and dissolved oxygen) individually controlled impellers, liquid reservoirs and syringe pumps, which make it possible for experiments to be set up and turned around rapidly. This high performance system enables rapid assessment of process parameters for optimum cell growth, expansion and cell quality [1].

The control software enables automation by managing all process parameters, including automation of feed additions. In this investigation, the software directs the pumps to deliver constant feeding into the vessel. Moreover, using an equation wizard, the control software calculates stir speed and gas flow (in particular air and CO_2) as a function of other critical variables (working volume and total gas flow percentage, respectively – Table 1).

This is one of the many software features that can allow better standardized control, for example, enabling automated feeding to save operator time.

Therefore the Ambr® software capabilities, in particular the equation wizard, enables R&D scientists to unveil relevant process information and utilise it in a highly consistent manner, while providing a system to include a variety of parameters in equations that can be used for further automation.

Figure 1. Single-use Ambr® 250 Modular bioreactor with integrated fluid supply system



Experimental Design

Unbaffled vessel: performance evaluation

The performance of the Ambr® 250 unbaffled (single impeller) was tested against the standard (baffled, twin impeller) vessel and spinner flask (control).

Process workflow

Following thawing, T cells were activated and transduced. On day 0, 0.3x10^6 T cells mL^{-1} were inoculated in the Ambr® 250 vessels and the spinner flask (control). Feeding, cell dilution and monitoring steps were carried out as outlined in Figure 3 and Table 1 below. DO was not controlled, just monitored.

Figure 2: Evaluation of Ambr® 250 unbaffled vessel versus the baffled vessel and control (spinner flasks).

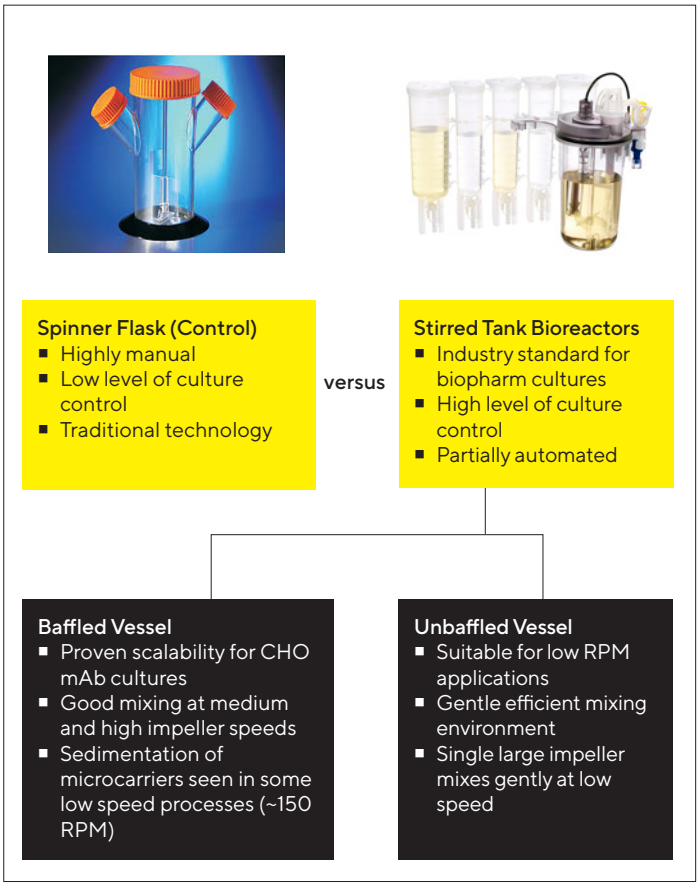


Figure 3: a) Typical CAR-T process. b) Experimental plan workflow

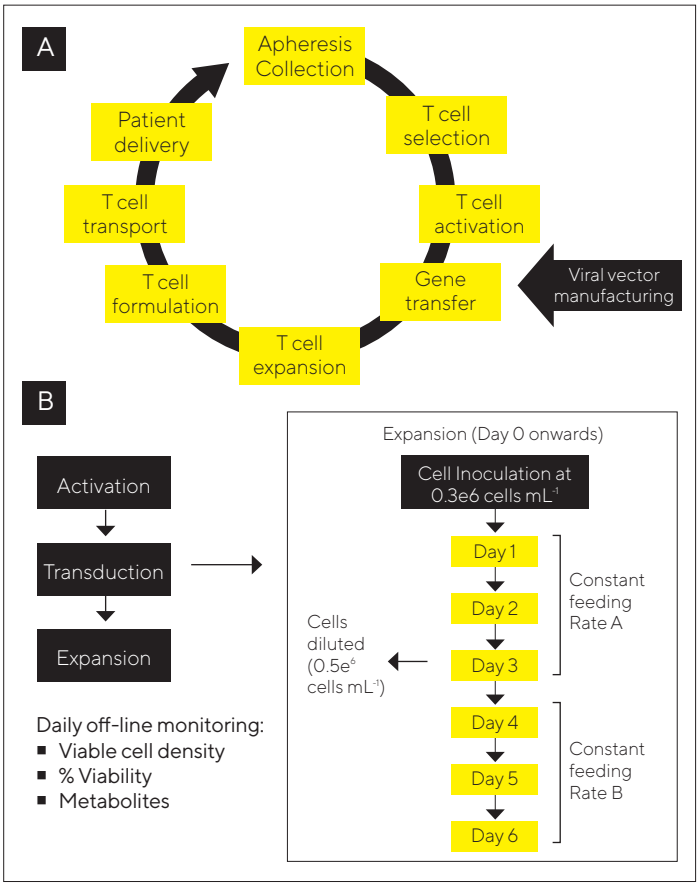


Table 1. Process parameters for Ambr® 250 runs

Process parameters used for both Ambr® 250 baffled and unbaffled vessels	
Working Volume	150 to 250 mL during process
Feeding strategy	25 mL day^{-1} (Feed Rate A) 40 mL day^{-1} (Feed Rate B)
Process Temperature	37°C
Gassing strategy	Headspace only. Air flow calculated based on working volume CO_2 added flow calculated (5% of air flow)
Gas flow	Air at 0.3 vvm
Stir strategy	Stir speed calculated based on volume
Stir speed	150 RPM
Stir direction	Upwards
DO strategy	Non-controlled, monitored only
pH set point	7.2 ± 0 .1

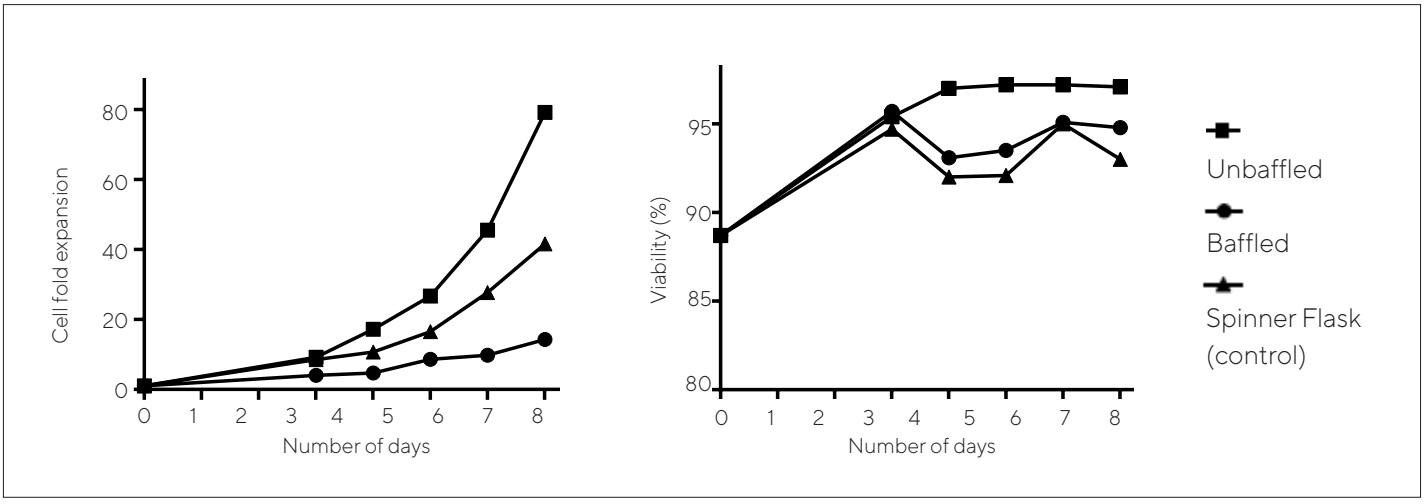
Results

Cell expansion, viability and phenotype

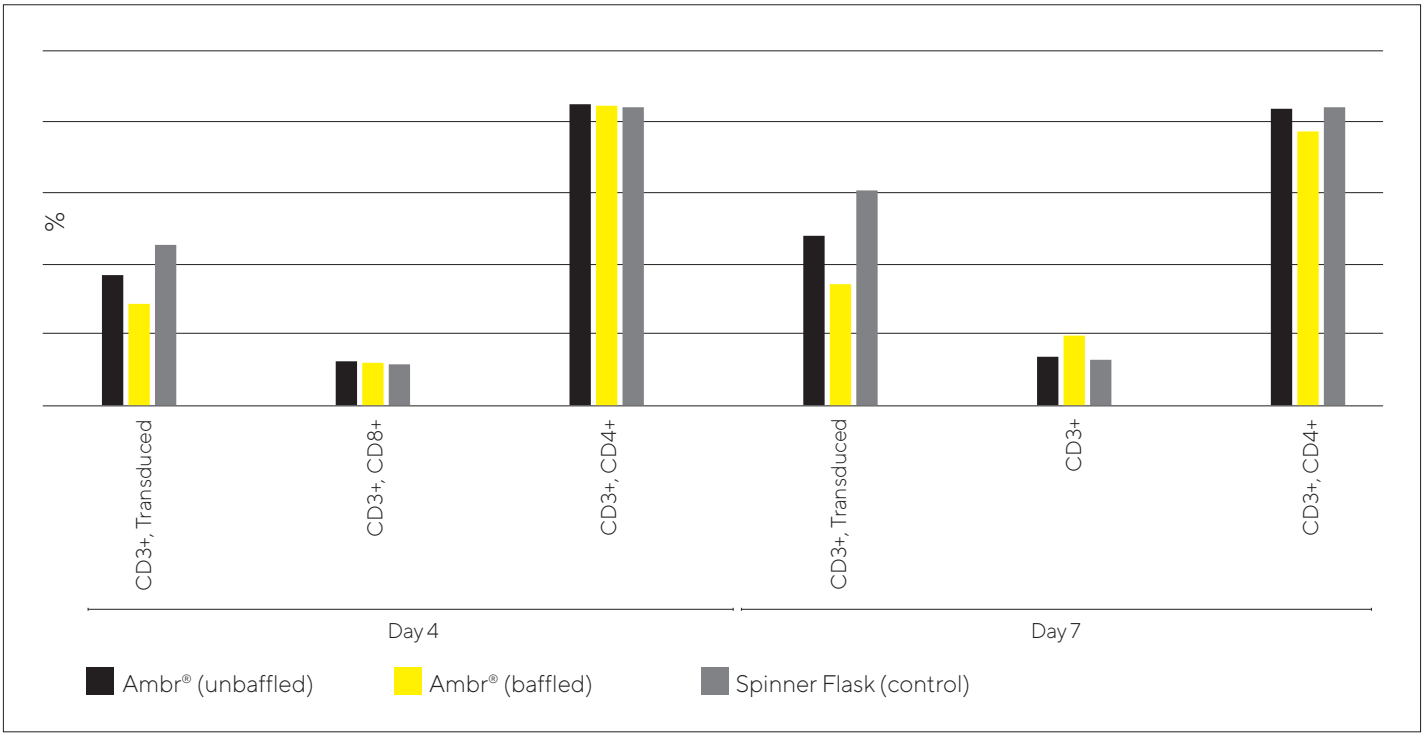
During the expansion phase day 0 onwards cell expansion and viability was measured in all culture systems using an automated cell counter. Results indicate better cell expansion in the unbaffled vessel, outperforming both the baffled vessel and the spinner flask (control), as shown in Figure 4a. The overall cell fold expansion was 1.9x higher for the unbaffled vessel showing exponential cell growth up to day 10. Viability was also higher throughout the expansion phase in the unbaffled vessel (Figure 4b). All vessels tested achieved comparable transduction efficiencies.

Cell phenotyping was carried out for the two Ambr® vessels and the spinner flask (Figure 4c), all vessels tested achieved comparable transduction efficiencies (data not shown). This indicates that the ambr vessels provide comparable quality of T cell as the spinner flask (control).

Figures 4a and 4b: Cell fold expansion and cell viability (%)



Figures 4c: Immunophenotyping for the 3 conditions explored.



Volume, pH and DO

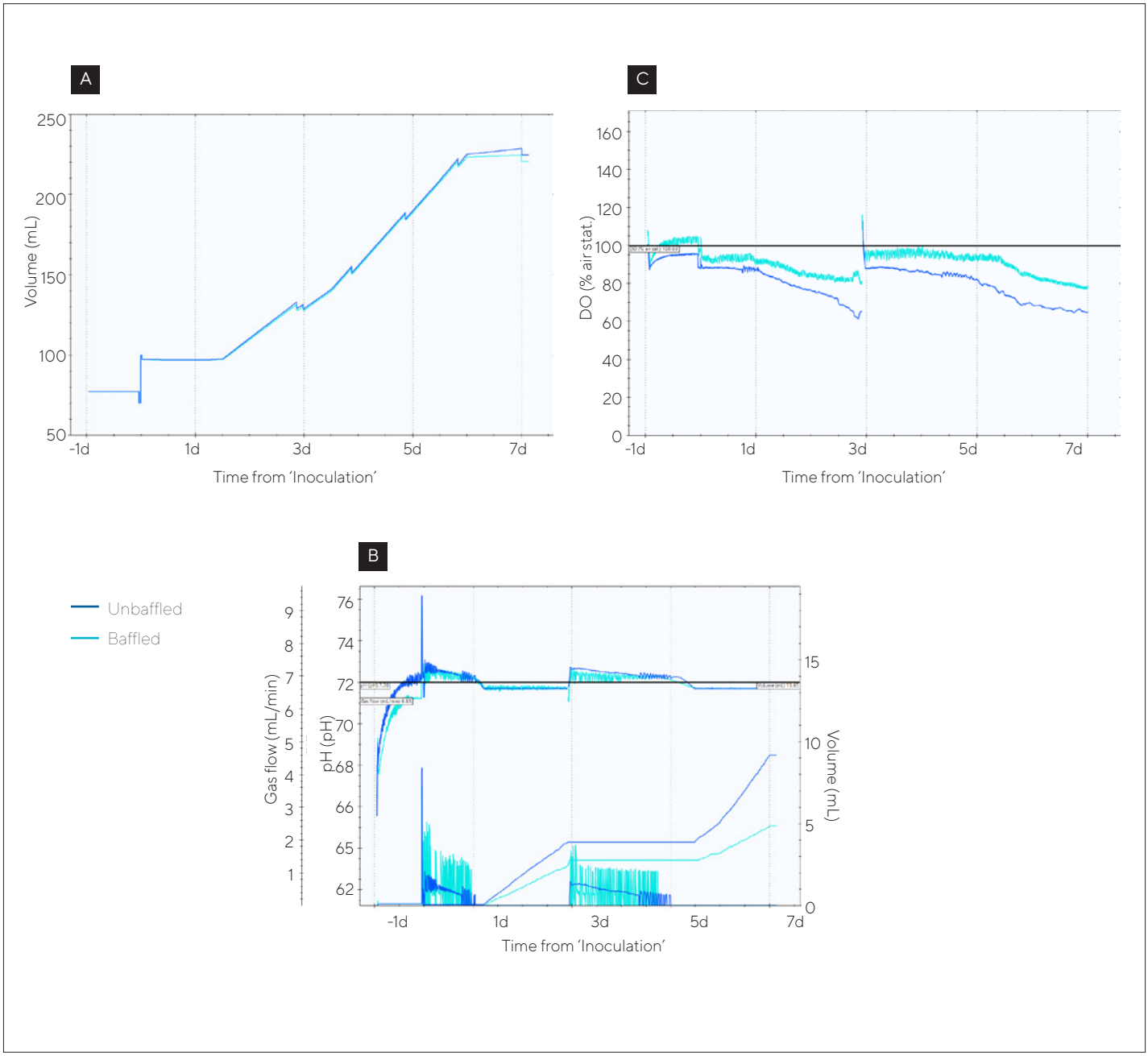
The automatic feeding (volume control, Figure 5a), described in the software protocol, performed as expected.

The Ambr® 250 pH automation uses CO_2 gas flow to control the upper pH band (7.2+0.1) and has allowed for good control before and after dilution (Figure 5b) and in both baffled and unbaffled vessels, independently of the different growth profiles. For the unbaffled vessel, as there was more cell growth observed, more lactate was produced by the cells and therefore more base was needed to balance the acidity, therefore controlling the lower pH band (7.2-0.1) .

As expected, the higher the T cell expansion, the lower the DO value (Figure 5c), reaching as low as 60% in the unbaffled vessel. DO profiles give further insights not previously observed using spinner flasks. This observation may lead to an investigation around whether or not there is a control strategy to be established in the future for enhanced process standardization.

It may be that establishing a controlled DO set point may be optimum for specific phenotype or other CQAs. Should DO be controlled in the future, this study may give an indication on possible set points to be further investigated.

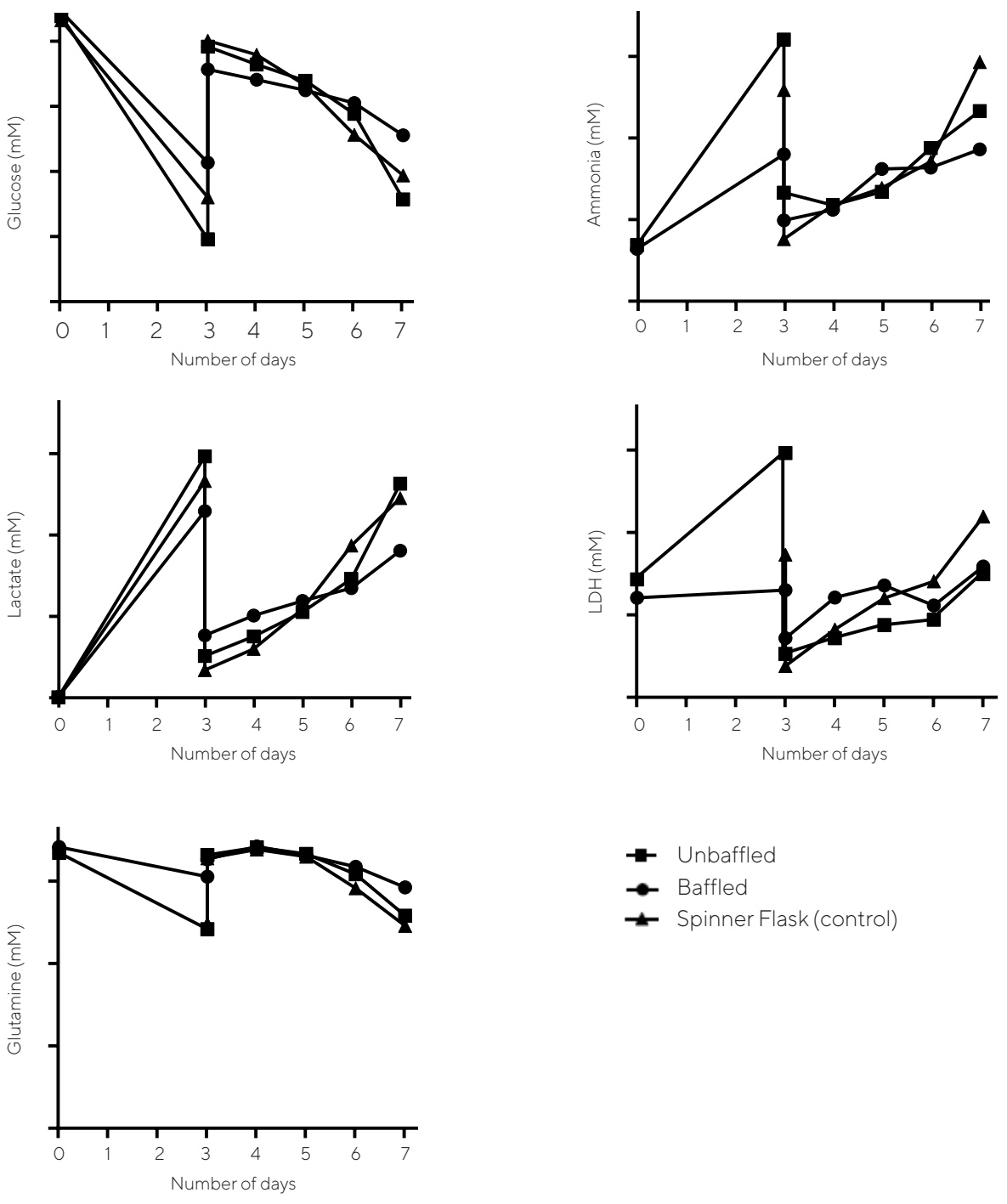
Figure 5: (a) Volume of fresh media added. (b) pH profile. (c) DO profile



Metabolites

Glucose and lactate as well as glutamine, ammonia and lactate dehydrogenase (LDH) profiles (Figure 6) showed that the unbaffled vessel conditions were comparable to the spinner flask (control) conditions.

Figure 6: Metabolite profiles for all three culture vessel types.



Discussions and Conclusions

Regarding the software, with the Ambr® 250 platform it is possible to easily set up parameters that are calculated as a function of other parameters, such as this study exemplifies: the CO_2 % added flow depends on the current bioreactor air flow at a particular given working volume. This enables investigations to include complex equations and allow R&D teams to delve further into their evaluation of critical parameters for cellular immunotherapy applications.

From a hardware perspective, the Ambr® 250 Modular single use vessel assembled with 5 reservoirs allows easy automated feed additions with integrated pumps and compact consumables that save time and effort during set up. The unbaffled Ambr® vessel yielded ~52% higher T cell expansion while maintaining key quality attributes similar to the control condition (spinner flask). Regarding the outcomes of the investigation, the Ambr® system allowed an increased understanding of the T cell DO behaviour. The increased cell expansion using the Ambr® unbaffled vessel option also provides not only an efficient system for process development but also an economically viable solution for in-depth studies of cellular immunotherapies.

For scale up, the automated Ambr® system enables advanced R&D process investigations to be easily transferred into larger bioreactor scales such as Biostat STR® while optimizing experimental conditions in a standardized manner across bioreactor platforms.

This study allowed the manufacturer of this cellular immunotherapy to better understand their process and get a feel for the large scope possible process improvements by further analysis and experimentation using the Ambr® 250.

Acknowledgments

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[1] Application note: Ambr® 250 High Throughput Microcarrier Cell Culture Processing

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