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APPLICATION NOTE

# Blue®Washer and Blue®Plate: Streamlining Labor-Intensive and Consumable-Heavy Flow Cytometry Screening Applications within Pre-Clinical CAR T cell Development

Wolfgang Mann[1], Shannon Frewen[1], Hanne Juchtmans[2]
[1]Blue Cat Bio GmbH, Neudrossenfeld, Germany
[2]Galapagos, Mechelen, Belgium

## BACKGROUND

Chimeric antigen receptor (CAR) T cell therapies have emerged as powerful tools in cancer treatment, particularly for hematological malignancies (Mitra et al., 2023). However, before these therapies reach the clinic, they must undergo rigorous preclinical research and development, a process in which flow cytometry plays a crucial role.

Flow cytometry is integral to CAR-T cell development, contributing to several key aspects. It facilitates the characterization of target cells by identifying and profiling antigens expressed on cancer cells. Additionally, flow cytometry quantifies CAR expression on engineered T cells, ensuring optimal receptor levels for effective targeting. It also assesses transduction efficiency by evaluating the success of gene transfer into T cells. Functional assays measure CAR-T cell functionality, assessing cytokine secretion, proliferation, and cytotoxicity against target cells. Moreover, flow cytometry serves as a quality control tool, monitoring cell viability, purity, and phenotype. Overall, flow cytometry is indispensable for the optimization and quality control of CAR-T cell therapy development (Sarikonda et al., 2021).

Flow cytometry, while essential, can be extremely time-consuming and resource-intensive. The process involves multiple washing steps and the use of numerous single-use pipette tips, significantly increasing both the time and cost associated with these experiments. This not only drives up costs but also contributes to considerable plastic waste. Such high consumption of resources is unsustainable in the long term, prompting many laboratories to seek more sustainable and cost-effective alternatives. By adopting automated systems like the Blue®Washer and Blue®Plate, labs can reduce their environmental footprint, decrease consumable costs, and streamline workflow efficiency, aligning with a growing emphasis on sustainability in scientific research.

The CAR-T cell screening team at Galapagos (Mechelen, Belgium) explored automation solutions to streamline and automate flow cytometry-based CAR-T cell screening applications. They found that up to 10 plates for various applications within CAR-T cell development need to be processed per screen/experiment leading to extensive hands-on labor and consumable usage. The main driver for looking into automation was to speed up these processes. Here, we report the results of using the Blue®Washer and Blue®Plate for flow cytometry sample preparation in two particularly labor- and time-intensive applications within the CAR-T cell screening process:

1. **B cell profiling:** B cell profiling is a crucial aspect of CAR-T cell development, serving multiple purposes. At Galapagos, this technique is utilized to evaluate the expression of specific target antigens on the surface of B cell lines and to determine the binding affinity of CAR constructs to these target antigens. This comprehensive approach ensures that CAR-T cells are effectively designed for their intended targets.
2. **Transduction efficiency in donor cells:** Determining Lentiviral transduction efficiency is essential to ensure the successful transfer of genetic material encoding the CAR to T cells. It is

crucial to quantify the percentage of T cells that successfully receive and express the CAR construct.

Utilizing the Blue®Washer and Blue®Plate significantly saved time and reduced the number of single-use pipette tips, resulting in substantial cost savings while maintaining data quality comparable to the conventional manual method. This success led Galapagos to adopt the Blue®Washer and Blue®Plate for these applications within the CAR development process and to explore further testing for additional applications. Additionally, the efficiency of the Blue®Washer dispensing system in effectively resuspending cells from the Cell®Safe of the Blue®Plate during wash cycles was tested. No notable differences between the four different Blue®Washer dispensing programs were observed. Consequently, the most efficient option was chosen by Galapagos for their standard protocol.

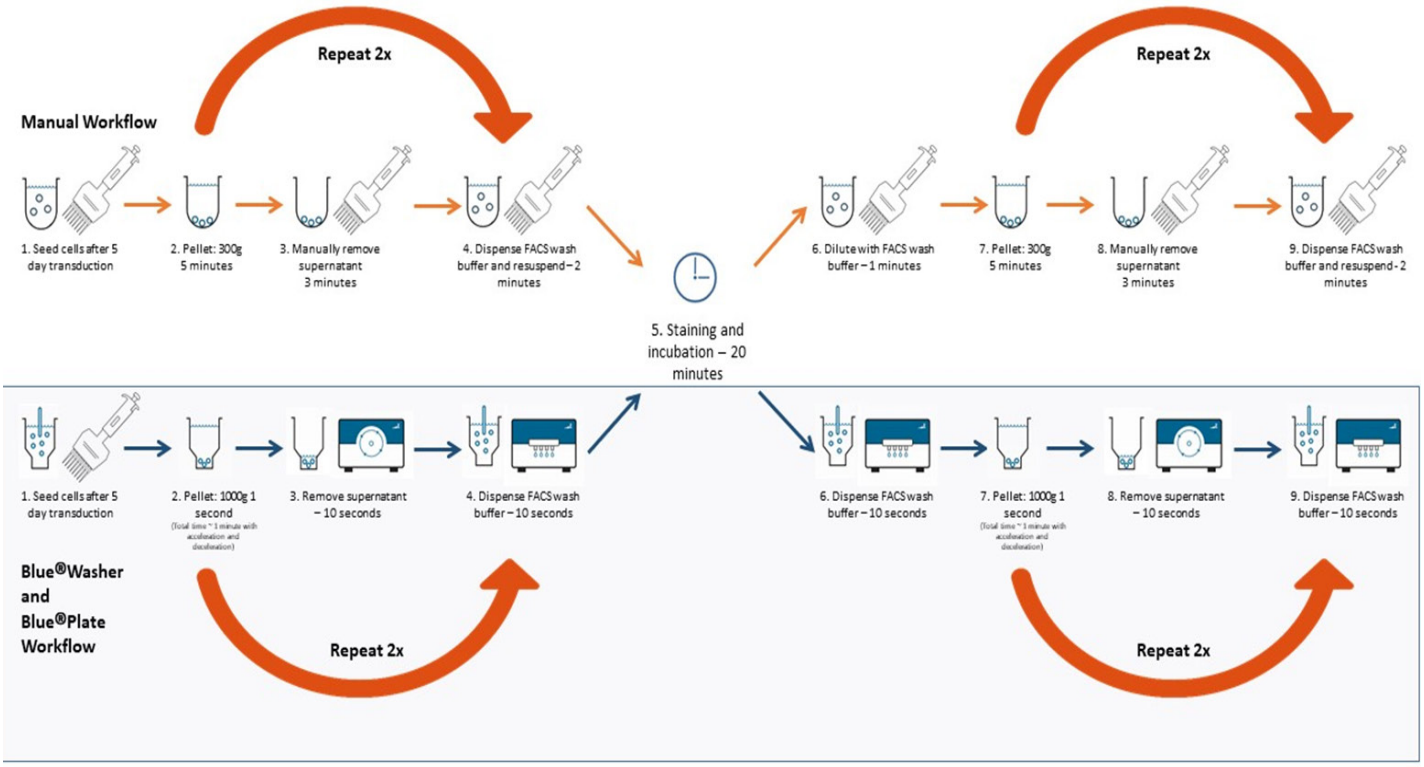


Figure 1. Simplified infographic of the Manual and Blue®Washer/Blue®Plate methods. Centrifugation/cell pelleting: 5 minutes, supernatant removal: 3 minutes, wash buffer addition and resuspension: 2 minutes. Blue®Washer wash times defined as 1 minute 20 seconds. Centrifugation/ cell pelleting: 1 minute (acceleration and deceleration of centrifuge requires time to reach speed ~30 seconds). Centrifugal evacuation: 10 seconds. Dispensing wash buffer: 10 seconds. Infographic gives a shortened view of full Galapagos method.

# METHODS

## B Cell Profiling

To evaluate the efficacy of the Blue®Washer and Blue®Plate in streamlining B cell profiling sample preparation, these devices were utilized to automate the cell washing steps prior to flow cytometry analysis. Five distinct B cell lines were assessed, with each cell type stained using one of six stains specific for six specific targets in addition to a live/dead stain and Fc block. The manual workflow was executed once, while the Blue®Washer was tested with four different dispense settings to examine potential effects on washing efficacy. Five B cell lines were seeded into either the Blue®Plate or a standard V-bottom 96-well microtiter plate. Old media was removed by washing the cells twice with PBS followed by the application of the live/dead stain. The wells were then washed twice with FACS buffer (comprising PBS, EDTA 1mM, and HSA 0.5%), before extracellular staining. After staining, the cells underwent two additional washes with FACS buffer before being analyzed using a BD Fortessa flow cytometer. Cells were directly acquired from the Blue®Plate utilizing the BD HTS AutoSampler.

## Transduction Efficiency

To assess transduction efficiency, cells isolated via leukapheresis from two donors were used. Isolated cells were stained with live/dead, CD3 and CD45 to select for T cells. In addition, samples were stained with one other stain either the target specific CAR stains (blinded in results) or CAR design specific stains (VHH and G4S). Each staining condition was tested once manually and replicated four times using the Blue®Washer and Blue®Plate, with four different dispense programs to evaluate potential differences in washing efficacy. Transduced cells were first washed twice manually with PBS in the original culture plate to ensure removal of remaining viral particles. After cells were efficiently washed, they were transferred into the Blue®Plate. This was followed by live/dead staining and two additional washes. After extracellular staining, the cells were washed twice more before analysis with the BD Fortessa flow cytometer. Cells were directly acquired from the Blue®Plate using the BD HTS AutoSampler in conjunction with the BD Fortessa Flow Cytometer.

Table 1. Description of different methods tested. Different Blue®Washer dispensing programs were trialed to determine whether cells were being efficiently resuspended and washed.

| Method | Supernatant removal | Wash buffer addition | Plate type |
|---|---|---|---|
| **Manual** | Multichannel pipette aspiration | Multichannel pipette aspiration 200ul | 96 well V bottom |
| **Blue®Washer / Blue®Plate Method 1** | Blue®Plate Evacuation program | Blue®Washer dispense: Double dispensing, 100ul Pressure 3 / 100ul Pressure 5 | Blue®Plate |
| **Blue®Washer / Blue®Plate Method 2** | Blue®Plate Evacuation program | Blue®Washer dispense: Double dispensing, 50ul Pressure 2 / 100ul Pressure 5 | Blue®Plate |
| **Blue®Washer / Blue®Plate Method 3** | Blue®Plate Evacuation program | Blue®Washer dispense: Double dispensing, 100ul Pressure 2 / 100ul Pressure 5, Rocking 20 seconds. | Blue®Plate |
| **Blue®Washer / Blue®Plate Method 4** | Blue®Plate Evacuation program | Blue®Washer dispense: 200ul Pressure 5 Straight head attachment | Blue®Plate |

# RESULTS

## Time Comparison

The Blue®Washer and Blue®Plate significantly decreased workflow time compared to the manual method, completing the entire workflow, encompassing six wash steps from cell seeding to flow cytometry analysis, in approximately 58 minutes. In contrast, the manual method required about 110 minutes to complete the same tasks. This marked reduction in processing time underscores the efficiency of automation in laboratory workflows, enabling researchers to increase throughput and allocate more time to critical analysis (Figure 2). Moreover, the automation eliminated the need for manual pipetting, except for the addition of antibody stains. This not only reduced the labor involved but also minimized potential sources of error.

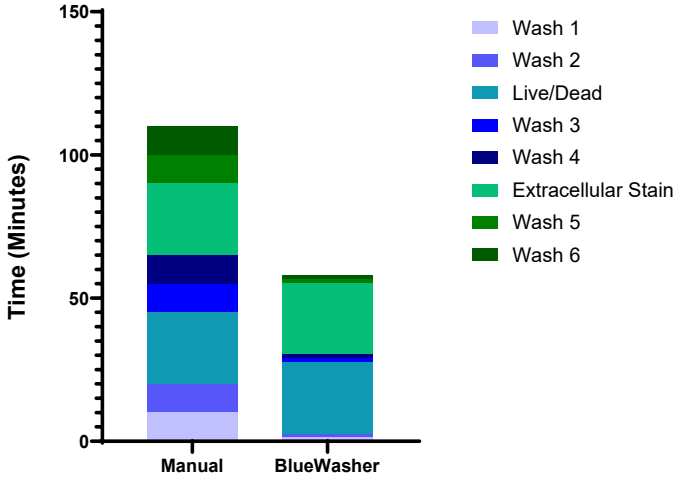


Figure 2. Manual versus Blue®Washer and Blue®Plate workflow time comparison. Manual wash times defined as 10 minutes. Blue®Washer and Blue®Plate wash times defined as 1 minute and 20 seconds. Staining steps are approximately 25 minutes (antibody addition: 5 minutes and incubation: 20 minutes).

## Consumable Cost Comparison

Total cost comparison between the manual and Blue®Washer/ Blue®Plate methods revealed a significant difference. Processing a single 96-well plate with the Blue®Washer/ Blue®Plate method incurred a cost of labor and materials of €250 vs €289 for the manual method (see Table 2).

More complex workflows will produce even bigger cost savings from reduced labor and pipette tips. In addition to cost savings, the simplicity of the Blue®Washer and Blue®Plate workflow, which eliminates the need for pipetting during washing steps, reduces the risk of human error and allows even new or inexperienced technicians to use the method quickly with minimal training making it easier to scale the throughput of flow cytometry analysis. Importantly, the Blue®Washer method also results in significantly less plastic waste.

Table 2. Comparative cost analysis of Blue®Washer and manual sample preparation methods. The prices shown are based on average market rates for each consumable, not specific to Galapagos. Fully allocated labor cost per hour is an estimate only.

| | Cost per Unit | Blue®Washer Method | | Manual | |
|---|---|---|---|---|---|
| **Consumable/Labor** | **EUR** | **Amount of units used in workflow** | **Cost (EUR)** | **Amount of units used in workflow** | **Cost (EUR)** |
| Pipette tips | 0,15 € | 192 | 28,80 € | 1344 | 201,60 € |
| U bottom microtiter plate | 4,00 € | | | 1 | 4,00 € |
| Blue®Plate | 180,00 € | 1 | 180,00 € | | |
| 1 hour of "Fully Allocated" Labor | 50,00 € | 50 minutes | 41,67 € | 100 minutes | 83,33 € |
| **Total price/sample.** | | | 2,61 € | | 3,01 € |
| **Total price/96 samples (1x 96 well plate)** | | | 250,47 € | | 288,93 € |

## B Cell Profiling

Measurement of Mean Fluorescence Intensity (MFI) values across all conditions revealed that the values obtained with the Blue®Washer and Blue®Plate were comparable to those achieved with the manual method. The Blue®Washer and Blue®Plate workflow did not compromise the quality of data obtained in terms of fluorescence intensity, demonstrating its reliability as a tool for streamlining and standardizing washing procedures in flow cytometry experiments.

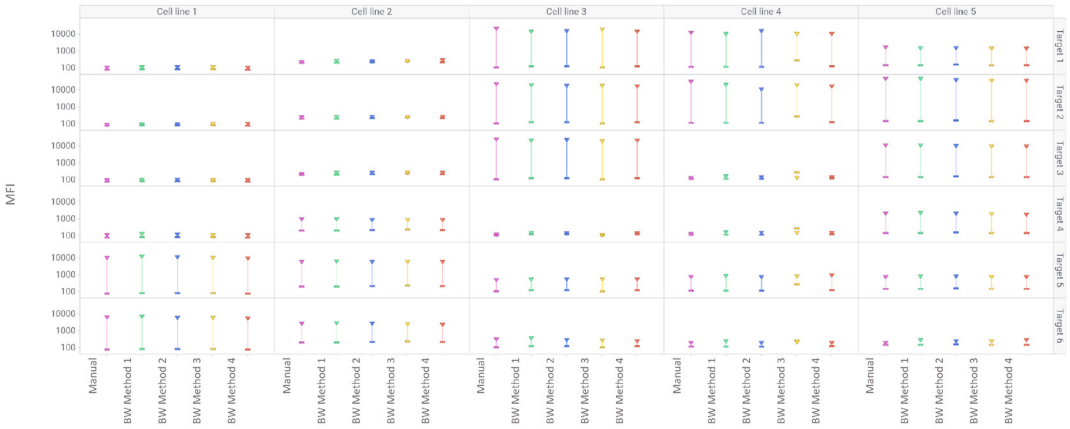


Figure 3.
The bottom bar represents cells stained only with the live/dead (L/D) stain, serving as the negative control for the target. Triangles indicate cells that are positive for the target antigen. All cell lines and target details are blinded to protect Galapagos' intellectual property.

## Transduction Efficiency

Transduction efficiency was assessed using isolated T cells from two different donors, targeting seven distinct CAR constructs. The results demonstrated comparable percentages of CAR-T cells between the two donors and across the manual and four different Blue®Washer and Blue®Plate methods, indicating no significant differences in staining. Although variability was observed for the VHH stain subsequent experiments confirmed that this was not attributable to the Blue®Washer and Blue®Plate method. This consistency underscores the reliability of the Blue®Washer in standardizing and automating washing procedures without compromising the quality of CAR T cell transduction outcomes.


Figure 4.
Percentages of CAR-T cells in two different donors. Seven distinct CARs were identified by their specific protein targets. G4S and VHH are not blinded as they are common stains used for CAR identification. All other information remains blinded due to Galapagos' intellectual property. UT indicates un-transduced cells. Some data for the VHH Manual Donor 1 and VHH/BW method 4 is omitted due to experimental issue unrelated to Blue®Washer and Blue®Plate. Thick dashed line = Average along all different methods. Thin lines = +/- 3x the standard deviation.

## CONCLUSION

The integration of the Blue®Washer and Blue®Plate into flow cytometry workflows for CAR T cell development at Galapagos has demonstrated substantial benefits in both efficiency and cost-effectiveness. The automation provided by this system significantly reduces the time required for sample preparation, with the Blue®Washer and Blue®Plate streamlining the washing process and cutting the time needed by over 52% compared to manual methods. This time-saving advantage not only enhances productivity but also alleviates the labor-intensive nature of traditional flow cytometry workflows.

Economically, the Blue®Washer and Blue®Plate method was more cost-effective due to the reduction in consumable costs. This translates into significant financial savings, which, coupled with the environmental benefits of reduced plastic waste, underscores the sustainable advantages of adopting automated solutions like the Blue®Washer. The ability to process large volumes of samples efficiently while maintaining data quality comparable to traditional methods highlights the effectiveness of the Blue®Washer and Blue®Plate in optimizing flow cytometry applications.

Overall, the successful implementation of these automated technologies at Galapagos not only supports the high-throughput demands of CAR T cell development but also aligns with broader goals of reducing operational costs and minimizing environmental impact. As a result, Galapagos plans to continue leveraging the Blue®Washer and Blue®Plate for current applications and explore their potential for future innovations in CAR T cell research and beyond.

## REFERENCES

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BlueCatBio GmbH
Hornungsreuth 22a
95512 Neudrossenfeld
Germany
info@bluecatbio.com

BlueCatBio NH Inc.
112 Etna Road
Lebanon, NH 03766
United States of America
info@bluecatbio.com

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