Performance Evaluation of CAR-T Cell Source Isolated and Activated T Cells Using the ADAM-CellT

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Introduction

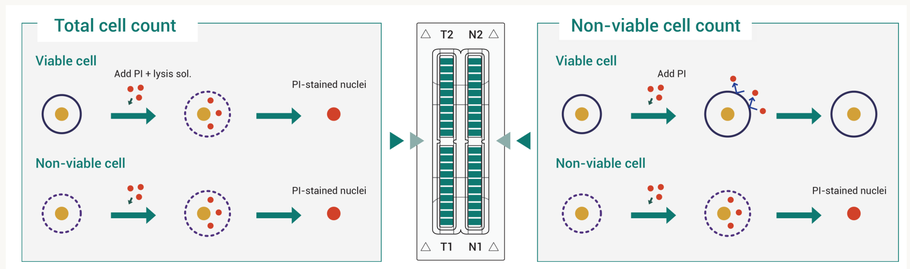
- Chimeric antigen receptor (CAR) T cell one of the immune cell therapy products, which is used for disease therapy such as cancer by overexpressing specific gene on T cells.
- Recently, CAR-T cells are being touted as the next generation cell to increase treatment efficiency[1].
- For that reason, the importance of monitoring of the intermediate products has come into the limelight[2].
- However, GMP facilities lack proper equipment which validates intermediate products, isolated or activated T cell, during CAR-T cell manufacturing processes[3].
- ADAM-CellT has been developed with the purpose of monitoring the quantity and viability of intermediate products in CAR-T cell manufacturing processes and also it is an optimized product for accurately and quickly counting isolated and activated T cells.

Methods and Procedures

- The precision, linearity, and method comparison of ADAM-CellT were evaluated in accordance with CLSI guidelines EP05-A3, EP06-A ,and EP09-A3.
- The isolated and activated T cells were made from human blood and were measured both with ADAM-CellT and by a comparative assay.
- The comparative assay was performed according to BD LeucocountTM Kit (BD biosciences; 340523) protocol using flow cytometry.

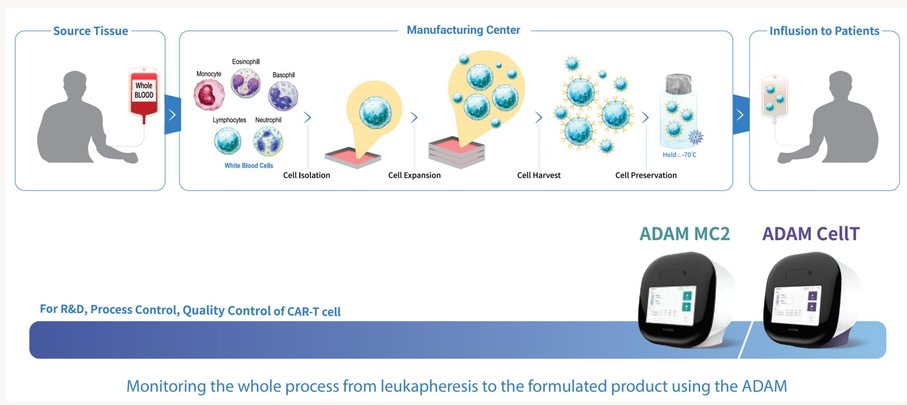
Optimization Workflow for Producing CAR-T

Principle of Viability Measurement (PI-staining Method)



* There are two types of disposable chips: 2 channel and 4 channel

QC Platform for Producing CAR-T



- It is easy to monitor all different steps of the purification, expansion, and formulation of CAR-T cells using the ADAM-MC2 and ADAM-CellT to ensure precise and reliable results. ADAM-MC2 and ADAM-CellT can be used for cGMP, process control and quality control of CAR-T cell manufacturing.

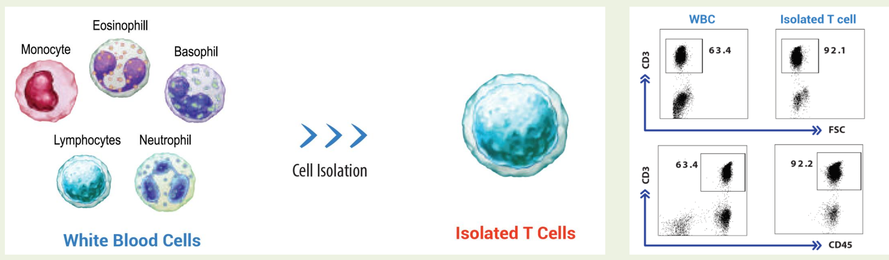
Conclusion

- Data indicates that the newly developed ADAM-CellT assay exhibits reliable performance. Consequently, we expect that ADAM-CellT will be a useful equipment to manage the monitoring of the quality of intermediate products during CAR-T cell manufacturing processes.

Results

- The Coefficients of variation of precision within each measuring series did not exceed 10% **(A, D)**.
- The analytical measurement range of the assays were 5 x 10^4 ~ 4 x 10^6 cell/mL with ordinary least squares regression fit of y = 1.0196x – 27468 (r^2=0.9962) **(B)**.
- In the method comparison studies with BD LeucocountTM Kit, the correlation coefficient (r) was 0.994, and the slopes/intercepts were 1.008 (95% CI= 0.9836 to 1.029)/-114.2(95% CI= -45499 to 26091) by Passing-Bablock regression fit **(C)**.

Performance Evaluation in Isolated T Cells



The profiles of T cells which was used for performance evaluation - Flow cytometric analysis of CD3 expression on unsorted (WBC; left panel) or sorted (Isolated T cell; right panel) human peripheral blood lymphocytes

A **Precision**

CLSI guideline EP05-A3

Level = Low

Level	Replicates	Mean	SD	CV
Low	20	486,336	24,181	4.97%

Level = Medium

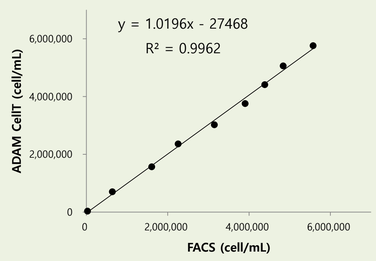
Level	Replicates	Mean	SD	CV
Medium	20	1,446,250	44,073	3.05%

Level = High

Level	Replicates	Mean	SD	CV
High	20	2,905,800	48,043	1.65%

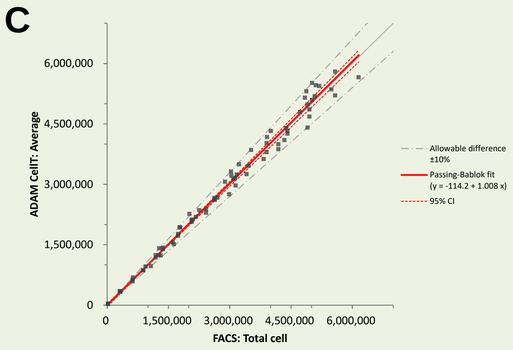
The sample that low, medium, and high concentration of isolated T cells were counted with ADAM-CellT

B **Linearity**



Comparison between flow cytometry and ADAM-CellT in Isolated T cells.

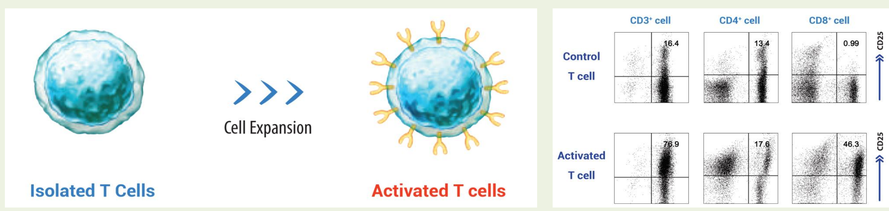
C



Sample size	79
y = -114.2 + 1.008 x	
Systematic differences	
Intercept A	-114.2
95% CI	-45499 to 26091
Proportional differences	
Slope B	1.008
95% CI	0.9836 to 1.029

Correlation of T cell counting counting between flow cytometry and ADAM-CellT in Isolated T cells.

Performance Evaluation in Activated T Cells



The phenotypes of activated T cells which was used for performance evaluation - Flow cytometric analysis of CD25 expression on TCR stimulated (Activated T cell; bottom panel) or unstimulated (control T cell; upper panel) T cells.

D **Precision**

CLSI guideline EP05-A3

Level = Low

Level	Replicates	Mean	SD	CV
Low	20	514,470	48,655	9.46%

Level = Medium

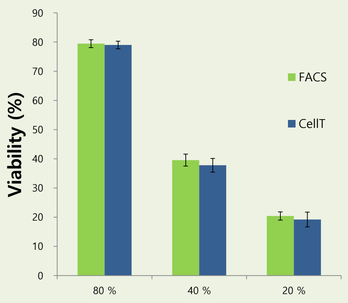
Level	Replicates	Mean	SD	CV
Medium	20	1,436,925	92,938	6.47%

Level = High

Level	Replicates	Mean	SD	CV
High	20	2,887,715	145,705	5.05%

The sample that low, medium, and high concentration of activated T cells were counted with ADAM-CellT

E



Comparison of viability between flow cytometry and ADAM-CellT in Activated T cells

References

1. Riddell SR. Cancer J. 20, 141-144 (2014).
2. Yonghong Li, *Engineering*. 5, 122-131 (2019).
3. Gee AP. *Best Pract Res Clin Haematol.* 31, 126-134 (2018).