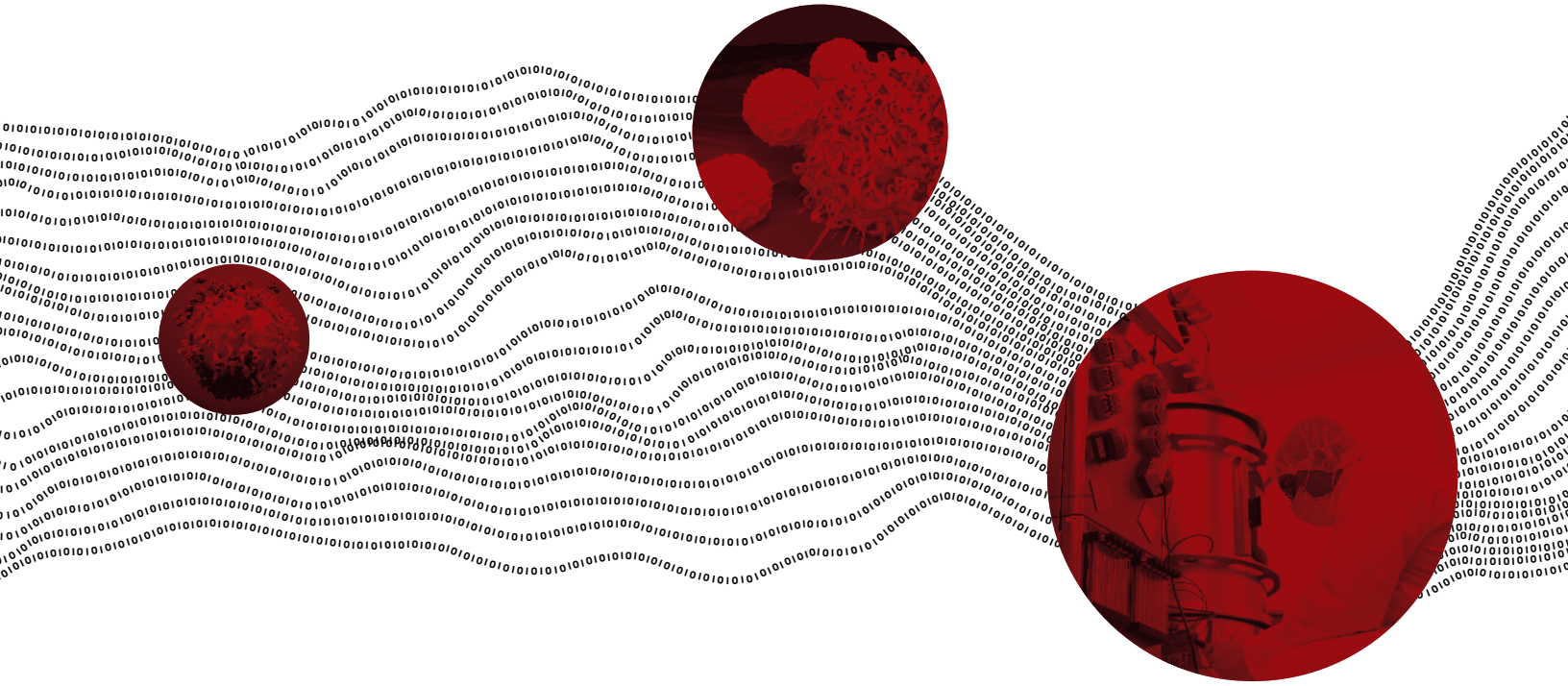
TECH NOTE

ASSAY REPRODUCIBILITY ON THE ACCELLIX PLATFORM



Accellix

INTRODUCTION

T cell therapies have shown tremendous promise for the treatment of historically difficult to treat cancers such as diffuse large B cell lymphoma, follicular lymphoma and acute lymphoblastic leukemia.

Engineered from the patient's own native T cell population, autologous T cell therapies provide a therapy option that is highly targeted, reduces treatment as well as patient recovery timelines, and can provide long-term benefit as engineered cells can persist in the body long after treatment is administered. T cell therapy manufacturing is a complex process wherein patient cell populations proceed through a multiphase cycle of extraction, characterization and isolation, optional genetic modification, cell expansion, harvest, and reinjection for treatment (Levine, Miskin, Wonnacott, & Keir, 2017). The process is cumbersome, lengthy, and highly variable based on the physiology of the patient cell population.

The manufacturing complexity and highly personalized nature of these "living therapeutics" greatly reduces the tolerable margin for error compared to more traditional cancer therapies. As such, for T cell therapies, quality control (QC) is one of the most critical elements. Errors in manufacturing that carry through the process can potentially be deleterious or even fatal for the patient. Fidelity of the cell population must be maintained continuously throughout the manufacturing cycle.

Existing QC tools for T cell manufacturing rely almost exclusively on conventional flow cytometry, however, these tools are becoming increasingly insufficient for the task (Campbell and Fraser, 2018). Conventional flow cytometry is difficult to scale for T cell therapies, because the instruments are expensive, require a large lab footprint and extensive technical training to operate. As well, conventional flow cytometry sample preparation, analysis, and data processing are time consuming and are very manual, increasing the likelihood of errors. These instruments are typically housed within centralized core labs far removed from the T cell manufacturing floor. Quality control must be performed at every step of the T cell manufacturing process, and a centralized model for QC adds an unnecessary layer of effort, time, and resources that can slow down the process, and potentially introduce new areas for user error. Ideally, flow cytometry instruments for cell therapy manufacturing QC should be compatible with the manufacturing suite. The Accellix Automated Cell Phenotyping Platform is available at the point-of-need, eliminates the limitations of conventional flow cytometry, and automates the entire QC workflow directly in the manufacturing suite. Small footprint bench-top platforms allow a decentralized approach to QC, with instruments available at each checkpoint in the manufacturing process, minimizing manufacturing downtime and maximizing speed to QC results. Smaller, dedicated QC platforms also offer minimal set-up, maintenance and user training times, and 24/7 accessibility that isn't supported by the centralized flow cytometry lab model.

Traditional therapeutic manufacturing—for example small molecules and antibodies—typically involves single, dedicated manufacturing sites at the center of large distribution networks. When therapeutic manufacturing is diversified among multiple sites, it is most often done to accommodate regionalization of distribution networks, but still generally involves a single manufacturing site in each region, typically delineated across large geographies, such as North America, the Eurozone, or Asia-Pacific, among others.

For cell therapies, manufacturing is shifting rapidly to a highly decentralized model, wherein designated regions of distribution are smaller and more numerous. This model situates therapeutic manufacturing suites closer to the patients, with whom the manufacturing process begins and ends.

This model of decentralization creates additional QC pressure for cell therapies. Regulatory expectations for

QC must be met across all manufacturing sites, and QC tolerances must be equivalent at all levels, within and among each manufacturing process, site, and region. To accomplish this, both accuracy and precision must be high and consistent for the chosen QC platform. Ideally, this means operating the same platform at all manufacturing sites, and here again, the limits of costly and bulky conventional flow cytometry technologies are evident.

Here we present data demonstrating consistent measurements of accuracy and precision for two of Accellix's off the shelf QC assays for T cell therapy products—Accellix T Cell Subset Assay and Accellix Lymphocyte Subset Assay, hereafter referred to as T Cell Assay and Lymphocyte Assay—performed on the Accellix Automated Cell Phenotyping Platform.

RESULTS

INSTRUMENT ACCURACY

To measure the accuracy of the Accellix platform, we compared measures generated with the T Cell and Lymphocyte assays to specifications associated with vendor-provided control blood.

We performed the T Cell assay on IMMUNO-TROL cells (Beckman Coulter), a positive process control sample for flow cytometry that enables detection of predetermined frequencies of immune cell populations. Fifty replicates of IMMUNO-TROL cells were run on an Accellix instrument and the resulting data analyzed to identify T cells that are CD45+ and CD3+ (Figure 1a). T cells were further subdivided into CD4+ and CD8+ populations, and frequencies of CD3+, CD4+ and CD8+ cells as a fraction of lymphocytes were reported. When we compared our measured cell frequencies to the range of values provided by the manufacturer, our values were well within the predicted value range, with coefficients of variation (CVs) ranging from 1.27 % to 2.91% amongst the 50 replicates (Figure 1b).

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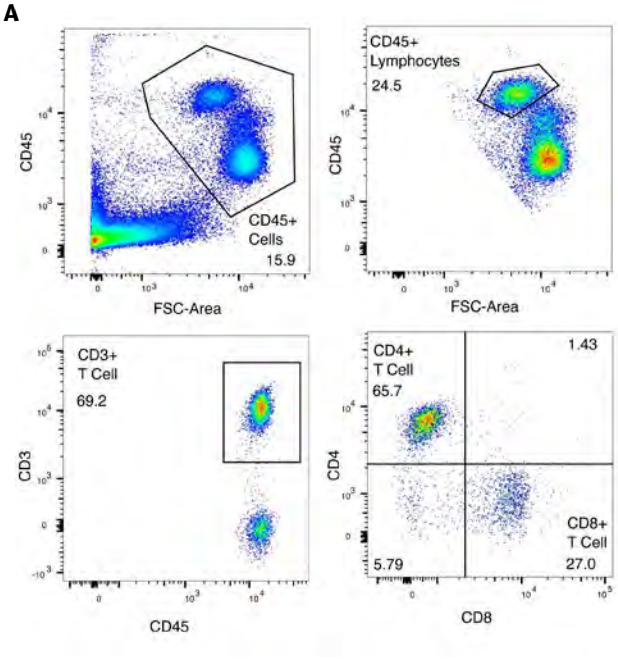

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	%CD3+ OF LYMPHOCYTES	%CD4+ OF LYMPHOCYTES	%CD8+ OF LYMPHOCYTES
Expected Range	67-85	39-57	21-33
Observed Mean	73.7	45.5	23.3
Observed Range	72.1-75.8	43.4-47.4	22-25.1
Standard Deviation	0.93	0.95	0.68
% CV	1.27	2.10	2.91

n=50

FIGURE 1. ACCURACY ASSESSMENT OF T CELL ASSAY.

(A) Representative flow cytometry plots showing gating of CD45+ cells, followed by gating of CD45+CD3+ cells. CD3+ cells are further subdivided into CD4+ and CD8+ cells. (B) Table shows expected ranges of cell subset frequencies in IMMUNO-TROL control blood, as well as observed mean frequencies, ranges, standard deviation and coefficients of variation (CV) observed on the Accellix platform.

We performed a similar study with the Lymphocyte assay on CD-CHEX Plus (Streck), another positive control for flow cytometric immunophenotyping. Forty-eight replicates of CD-CHEX Plus cells were assayed on an Accellix instrument and the resulting data analyzed to identify CD45+ cells that were CD14+ monocytes or CD14- lymphocytes (Figure 2a). The CD14- lymphocyte population was further subdivided into CD3+ T cells and CD19+ B cells. The CD14- lymphocyte population was also used to measure frequencies of Natural Killer (NK) cells, by gating on cells that are positive for CD56 or CD16, which were both analyzed in the same fluorescent channel, and negative for CD3. Frequencies of CD14+ monocytes, and CD3+ T cells, CD19+ B cells, and CD3-CD56/CD16+ NK cells as a fraction of lymphocytes were reported. When compared to the range of values provided by the manufacturer, our measured values again fell well within the predicted value range, with CVs ranging from 0.60% to 5.4% amongst the 48 replicates (Figure 2b).

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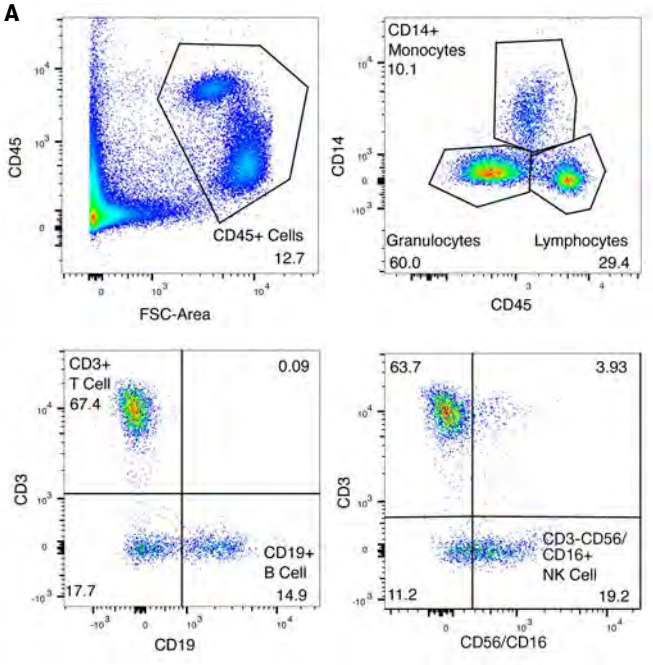

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	%CD3+ OF LYMPHOCYTES	%CD19+ OF LYMPHOCYTES	%CD14+ OF MONOCYTES	%CD3-CD56/ CD16+ OF LYMPHOCYTES
Expected Range	68.4-88.4	6-16	70-100	4.6-14.6
Observed Mean	75.3	10.5	89.1	11.7
Observed Range	74.1-76.1	9.19-11.8	83.7-95.5	10.7-12.8
Standard Deviation	0.45	0.57	2.99	0.45
% CV	0.60	5.40	3.36	3.92

n=48

FIGURE 2. ACCURACY ASSESSMENT OF LYMPHOCYTE ASSAY.

(A) Representative flow cytometry plots showing gating of CD45+ cells, followed by discrimination of CD14+ monocytes (CD14+CD45med), granulocytes (CD14-CD45low) and lymphocytes (CD14-CD45high). Lymphocytes were further subdivided into CD3+ T cells and CD19+ B Cells. Lymphocytes were also gated on CD3-CD16/CD56+ NK cells. (B) Table shows expected ranges of cell subset frequencies in CD-CHEX Plus control blood, as well as observed mean frequencies, ranges, standard deviation and coefficients of variation (CV) observed on the Accellix Platform.

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INSTRUMENT PRECISION

The second critical validation characteristic that must be analyzed when assessing an instrument is precision, which describes the closeness between a series of measurements obtained from multiple samplings of an analyte. Precision can be measured in two different methodologies: intra-assay precision or repeatability, and intermediate precision. Repeatability describes the degree of variance between results obtained from separate assays performed by the same operator on the same measuring system in the same lab. Intermediate precision describes the degree of variance between results obtained from separate

assays within the same laboratory, across different instruments. To measure repeatability on the Accellix platform, we analyzed the performance of the T Cell and Lymphocyte assays across multiple days. In the inter-day precision T Cell study, 5 replicates of IMMUNO-TROL cells per day were assayed on a single Accellix instrument. This was repeated for 5 consecutive days and the resulting frequencies of CD3+, CD4+, and CD8+ cells as a fraction of lymphocytes were compared amongst the assays. For each cell population, we observed strong concordance between measurements made on different days, with standard deviations under 1 and % CVs ranging from 1.33% to 3.03% (Figure 3a). We performed a similar analysis using the Lymphocyte assay on CD-CHEX Plus control cells and observed similar results. When comparing the frequencies of CD14+ monocytes, and CD3+, CD19+, and CD3-CD56+/CD16+ cells as a fraction of lymphocytes amongst the assays, we observed standard deviations ranging from 0.4 to 2.58 and % CVs ranging from 0.52% to 4.44% (Figure 3b).

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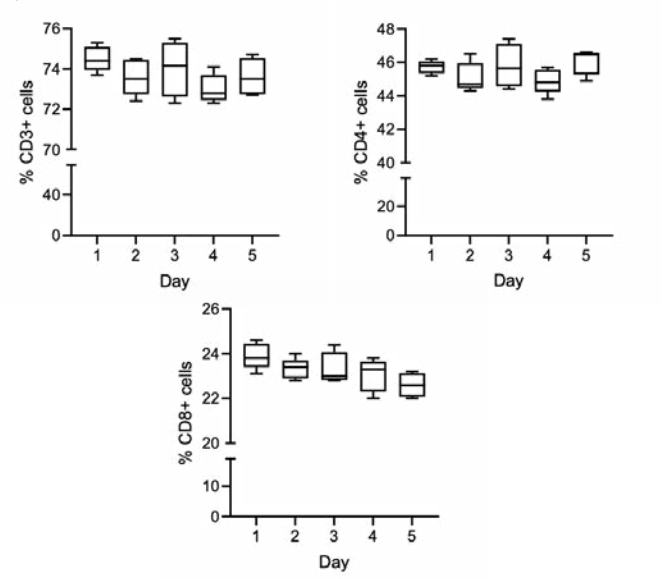

	%CD3+ OF LYMPHOCYTES	%CD4+ OF LYMPHOCYTES	%CD8+ OF LYMPHOCYTES
Mean	73.7	45.5	23.3
Standard Deviation	0.98	0.90	0.71
% CV	1.33	1.97	3.03

n=25

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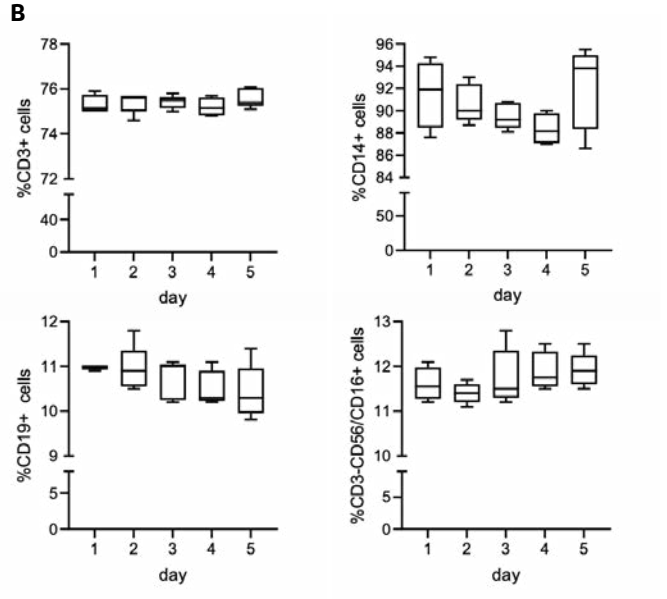

	%CD3+ OF LYMPHOCYTES	%CDI4+ OF LYMPHOCYTES	%CDI9+ OF LYMPHOCYTES	%CD3-CD56/CDI6+ LYMPHOCYTES
Mean	75.4	90.5	10.7	11.7
Standard Deviation	0.40	2.58	0.48	0.44
% CV	0.52	2.85	4.44	3.75

n=25

FIGURE 3. REPEATABILITY ASSESSMENT OF THE ACCELLIX PLATFORM.

A) T Cell Assay Graphs show the same sample run 5 times/day on one instrument across 5 days. Box and whisker plots show mean frequencies of CD3+, CD4+, and CD8+ T cells as a fraction of lymphocytes. Error bars denote 95% confidence intervals. Summary table shows mean, standard deviation, and CV for each population across the dataset. B) Lymphocyte Assay Graphs show the same sample run 5 times/day on one instrument across 5 days. Box and whisker plots show mean frequencies of CD14+ monocytes, and CD3+, CD19+, and CD3-CD56/CD16+ cells as a fraction of lymphocytes. Error bars denote 95% confidence intervals. The summary table shows mean, standard deviation, and CV for each population across the dataset.

To enable decentralized manufacturing of cell therapy products, both within the manufacturing suite and across healthcare regions, there must be confidence that each individual instrument, regardless of its location, is operating similarly to all of the other instruments in the fleet. To this end, we explored the closeness of measurements obtained from multiple instruments to assess inter-instrument precision. For the T Cell assay study, 65 replicates of IMMUNO-TROL cells were analyzed across 11 Accellix instruments and the resulting frequencies of CD3+, CD4+, and CD8+ cells as a fraction of lymphocytes were compared amongst the assays. We observed a strong correlation between measurements performed on different instruments, with standard deviations under 2 and CVs ranging from 2.04 % to 4.95 % (Figure 4a). We performed a similar study using the Lymphocyte assay, assaying 25 samples across 8 instruments. When the measured frequencies of CD14+ monocytes, and CD3+, CD19+, and CD3-CD56/CD16+ cells as a fraction of lymphocytes were compared across Accellix instruments, we observed standard deviations under 2 and CVs that ranged from 2.15% to 8.38% (Figure 4b).

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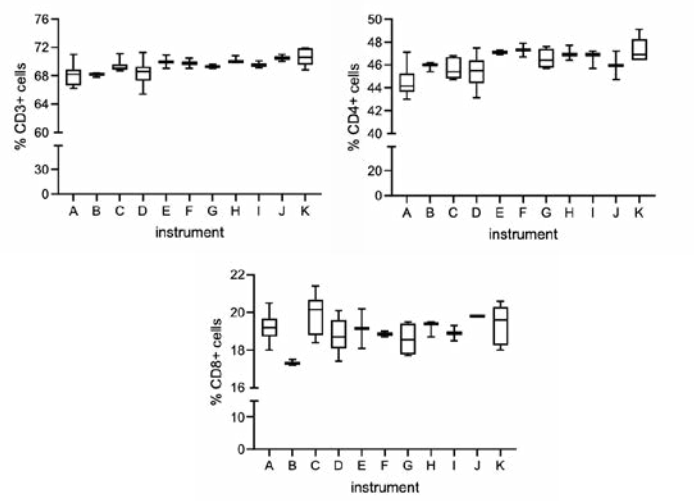

	%CD3+ OF LYMPHOCYTES	%CD4+ OF LYMPHOCYTES	%CD8+ OF LYMPHOCYTES
Mean	68.98	45.78	19.0
Standard Deviation	1.41	1.36	0.94
% CV	2.04	2.96	4.95

N=65 across 11 instruments

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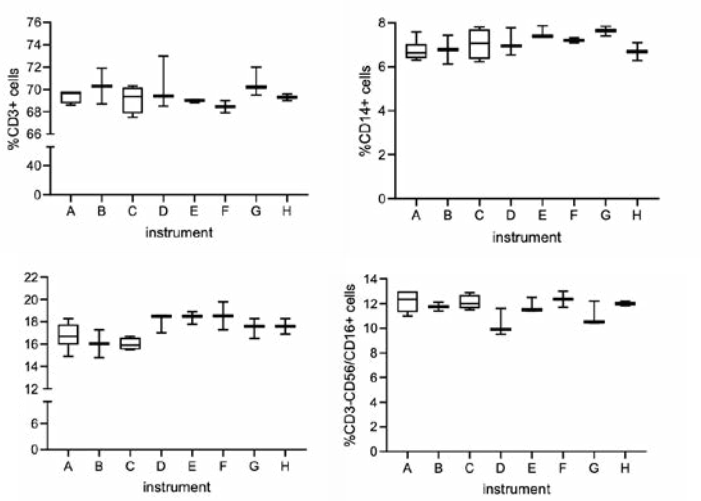

	%CD3+ OF LYMPHOCYTES	%CDI4+ OF LYMPHOCYTES	%CDI9+ OF LYMPHOCYTES	%CD3-CD56/CDI6+ LYMPHOCYTES
Mean	69.61	7.07	17.10	11.73
Standard Deviation	1.50	0.56	1.43	0.94
% CV	2.15	7.92	8.38	8.05

N=25 across 8 instruments

FIGURE 4. INTERMEDIATE PRECISION ASSESSMENT OF THE ACCELLIX PLATFORM.

A) T Cell Assay Graphs show 65 samples run across 11 instruments. Box and whisker plots show mean frequencies of CD3+, CD4+, and CD8+ T cells as a fraction of lymphocytes. Error bars denote 95% confidence intervals. Summary table shows mean, standard deviation, and CV for each population across the dataset. B) Lymphocyte Assay Graphs show 25 samples run across 8 instruments. Box and whisker plots show mean frequencies of CD14+ monocytes, and CD3+, CD19+, and CD3-CD56/CD16+ cells as a fraction of lymphocytes. Error bars denote 95% confidence intervals. The summary table shows mean, standard deviation, and CV for each population across the dataset.

CONCLUSIONS

The data presented here demonstrate a high degree of accuracy and precision for both the T Cell and Lymphocyte Assays measured on the Accellix Cell Phenotyping Platform.

Confidence values measured for both repeatability and intermediate precision were significantly below the accepted threshold for cell therapy method development of 15%. The stability of the platform demonstrated here, represented by the combination of these three measures—accuracy, repeatability, and intermediate precision—makes it ideal for rapid and consistent QC operations in a decentralized cell therapy manufacturing model.

Beyond the advantages noted earlier, a decentralized model for manufacturing QC supported by Accellix's cartridge-based QC platform offers additional benefits over the conventional centralized model. Sample preparation can be accomplished in a microfluidic cartridge containing room temperature-stable, dried reagents. Minimal sample volume is needed and the single-use cartridge eliminates the risk of cross-contamination. Critically, the point-of need, automated approach to QC can reduce QC workflow times by up to 80%, while maintaining continuity in the cell therapy manufacturing workflow itself.

METHODS

T CELL SUBSET ASSAYS

IMMUNO-TROL (Coulter) is an assayed, lysable whole blood quality control product for immunophenotyping analysis. IMMUNO-TROL cells were assayed using a standard Accellix T Cell Subset Reagent Tube Format (RTF) Assay kit with lysis buffer (T Cell RTF Assay (L) and following the manufacturer's instructions for use. Each Accellix RTF Assay kit includes a microfluidic cartridge and a unitized, dried reagent tube containing antibodies, viability dye, and control beads. The included antibodies are CD45-AF488, CD8-PECy5, CD4-PE, CD8-PED594, and DiYO-3 viability dye. Briefly, 40 µl of IMMUNO-TROL cells were transferred into the tube and mixed on a pulsed vortex mixer for 2 minutes. After mixing, 20 µl of sample mixture were transferred into the loading port of the RTF cartridge. The cartridge was inserted into the Accellix instrument and the assay run initiated.

LYMPHOCYTE SUBSET ASSAYS

Inter-instrument precision studies with the Lymphocyte Subset assay were performed on IMMUNO-TROL cells, as described above. We used the Accellix TBNK-16 assay kit with lysis buffer (TBNK-16 RTF Assay (L)), which includes CD45-PECy7, CD3-PECy5, CD14-PED594, CD16-AF488, CD19-PE, CD56-AF488, and DiYO-3 viability dye. Accuracy and inter-day precision studies were performed on CD-Chex Plus (Streck), which is also a positive control sample for immunophenotyping by flow cytometry. CD-Chex Plus cells were handled similarly to IMMUNO-TROL cells, described above.

CATALOG NUMBER	ASSAY NAME	DESCRIPTION	CONTENTS
A1000-1L	T Cell RTF Assay (L)	Full T Cell panel with lysis buffer for apheresis or samples requiring RBC lysis	CD45-AF488, CD3-PECy5, CD4-PE, CD8-PED594, DiYO-3 Viability, Control Beads
A1003-1L	TBNK-16 RTF Assay (L)	Expanded lymphocyte subset panel with CD16 antibody and lysis buffer for apheresis or samples requiring RBC lysis	CD45-PECy7, CD3-PECy5, CD14-PED594, CD16-AF488, CD19-PE, CD56-AF488, DiYO-3 Viability, Control Beads
IPT-2022	Accellix Integrated Cartridge Platform	Accellix instrument; includes 1 year warranty and installation at customer site	Instrument

REFERENCES

Levine, B. L., Miskin, J., Wonnacott, K., & Keir, C. (2017, March 17). Global Manufacturing of CAR T Cell Therapy. *Molecular Therapy*. Methods & Clinical Development, 4, 92-101.

Campbell, J and Fraser,A. (2018, September 21). Flow cytometric assays for identity, safety and potency of cellular therapies. Clinical Cytometry, 94, 725-735.

To learn more about the Accellix Cell Phenotyping Platform, email us at **info@accellix.com**

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