B33

CROSS INSTRUMENT STANDARDIZATION – A CORNERSTONE OF MEASUREMENT ASSURANCE

Dominic Gagnon, Jonathan Berthiaume, Marie Champoux, Audrey Gagnon, and Virginia Litwin

CAPRION

INTRODUCTION

Flow cytometry has become an integral component of the drug discovery and development process as novel therapeutic approaches targeting the immune system are being applied in oncology and infectious diseases. Given that flow cytometry data are being submitted to regulatory agencies to support biological license applications, it is important to ensure that measurements are made precisely and with certainty.

Flow cytometry data from clinical evaluation, as well as the manufacturing process for cellular therapies, are typically generated on multiple instruments often in multiple laboratories. Thus, it is critical that instruments are cross-standardized.

Several approaches for instrument cross standardization have been reported in the literature. In this poster we will present data from two approaches to instrument standardization of BD LSRFortessa™ and discuss the advantages and disadvantages.

METHODS

Instrument Standardization Method 1

In this process, optimal assay specific instrument settings are established on the primary instrument which were established during the assay development/ optimization phase. Next, using optimally set experiment on the first instrument, target values for each detector are determined using CompBeads stained with each mAb in the assay. The same CompBeads are then used to transfer the target values to additional instruments in the same laboratory or across sites. Using the "Application Settings", similar MFI will then be maintained on both systems.

Instrument Standardization Method 2

With the second process, voltage titration experiments using unstained cells combined with mid-range FL1 Rainbow Florescent Particles were performed on all instruments in order to establish the optimal voltages for each PMT, i.e. the lowest voltage where the maximal stain index is achieved. Optimal setting for standardization was then established from the "poorest" performing cytometer in order to ensure that all instruments would meet the criteria. Then, using single stained PBMC, the positive signal was evaluated to ensure that it would be maintained within linear range. Using CompBeads for each fluorophore, these target values were then transferred to all instruments.

RESULTS AND DISCUSSION

Using the first approach, inter-instrument variation as low as 1 %CV was achieved in multiple studies (Figure 1). It's a rapid way to perform inter-instrument but can become quite heavy when multiples instruments and sites are to be set identically and regularly. A limitation of this approach is also that dim fluorescence signals can be more variable than the brighter signals.

Full implementation of the second approach is ongoing. Two instruments have been qualified. The first one has been fully tested using the voltage titration (Figure 2). An optimal stain index was calculated for each PMTs (Figures 3 and 4). Most of the data will display a robust Standard deviation (rSD) of the unstained cells that is three times the noise rSD. The two instruments were then set based on target values using CompBeads (Figure 5) and stained cells were acquired to compare instrument performance (Figure 6). This approach considers the performance of all cytometer and is not panel dependent. One advantage of this approach is that better standardization of dim signals was achieved. Also, there is no need to do a voltage titration antibody specific for each PMT. A disadvantage is that the approach is labor intensive to setup and may require unique standardization for some assays.

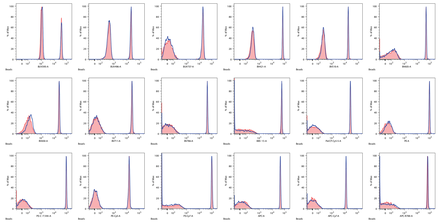

Figure 1: Instrument Standardization Method 1

The primary instrument (001, in red) was used to define target values that were transferred to the second cytometer (002, in blue). Mostly the data from both instruments are identical with CV's as low as 1%. Slight variations in the dim signals for some parameters (BV650, PE, and APC-R700) were observed.

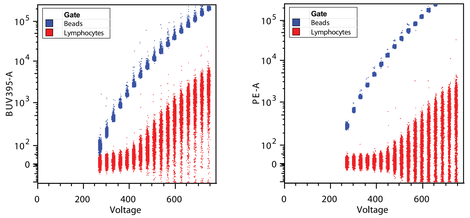

Figure 2: Instrument Standardization Method 2

Voltage titration to determine the Optimal PMT setting using unstained PBMC and mid-range FL1 beads. Voltage was change from 270 to 750 volts by increment of 30 volts. Median fluorescence of lymphocytes (in red) and beads (in blue) were recorded as well as rSD of lymphocytes. On the Left, BUV395 parameter testing, on the right, PE parameter testing. Note that for the last few voltages tested, beads are off scale.

Voltage	Beads BUV395- A Median	PBMC BUV395- A Median	PBMC BUV395- A rSD	SI	SI (% of Max)
270	107	1	15	3.5	5%
300	236	3	15	7.8	11%
330	481	7	15	15.8	23%
360	923	15	16	28.4	41%
390	1692	27	18	46.3	66%
420	2943	48	26	55.7	80%
450	4962	83	36	67.8	97%
480	8116	135	57	70.0	100%
510	12944	219	88	72.3	103%
540	20048	335	139	70.9	101%
570	30323	511	205	72.7	104%
600	44718	753	306	71.8	103%
630	64814	1086	441	72.3	103%
660	92219	1545	626	72.4	103%
690	129393	2162	905	70.3	100%
720	179245	2959	1278	69.0	99%
750	242819	3913	1757	68.0	97%

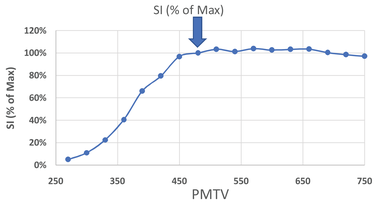

Figure 3: Instrument Standardization Method 2

Optimal PMT setting using unstained PBMC and mid-range FL1 beads. Voltage was changed from 270 to 750 volts by increment of 30 volts. Median fluorescence of PBMC and beads were recorded as well as rSD of cells. Stain Index (SI) = (Median of Positive (beads) - Median of Negative (PBMC)) / (rSD of Negative * 2) was calculated and the optimal voltage was found for the BUV395 parameter (480 volts, beginning of SI plateau).

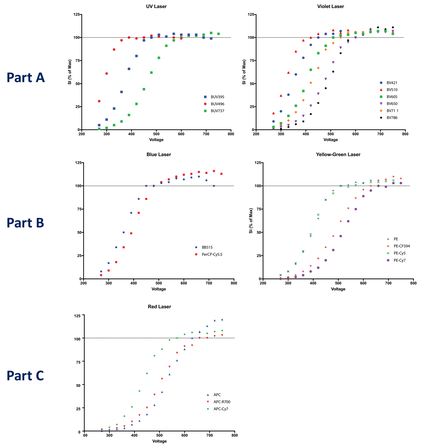

Figure 4: Instrument Standardization Method 2

Optimal PMT setting using unstained PBMC and mid-range FL1 for all PMT. Voltage was changed from 270 to 750 volts by increment of 30 volts. Median fluorescence of PBMC and beads were recorded as well as rSD of cells. Stain Index (SI) was calculated and the optimal voltage was found for all parameters (when the SI is at around 100% for the first time). From left to right, UV, Violet, Blue, Yellow-green, and Red laser parameters.

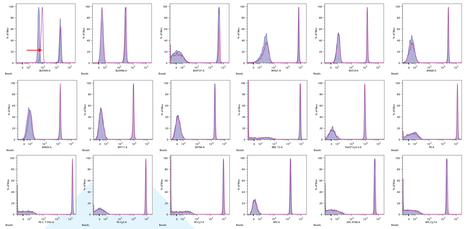

Figure 5: Instrument Standardization Method 1

Inter-instrument based on optimal PMT setting using single stained CompBeads. The primary instrument (002, in blue) was used to define target values that were set on the second cytometer (005, in pink). Note major variations in background for BUV395 parameter.

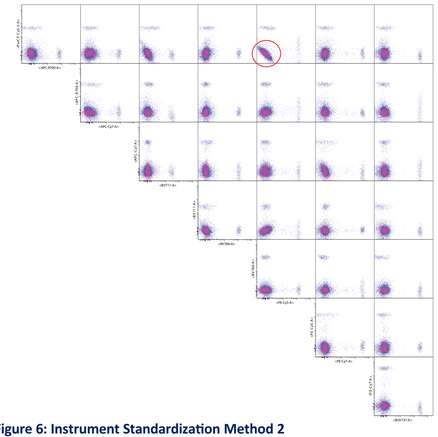

Figure 6: Instrument Standardization Method 2

Inter-instrument based on optimal PMT setting using single stained PBMC with CD4 for all 18 parameters then mixed together before acquisition. Overlay of the the primary instrument (002, in blue) with the second cytometer (005, in pink). Note, only end of spectrum for all laser was displayed in the NxN plot to show good resolution. Note also the strong compensation between PE-Cy5 and PerCP-Cy5.5 distorting the double negatives cells.

CONCLUSIONS

The next steps will be to continue the second approach for all our instruments across sites. This will inform us on best and poorer performer per PMT to set all instrument accordingly. We will also evaluate the double stained spill matrix to guide us in optimal panel design.

Given that inter-instrument variation is a major contributor to data variability, instrument standardization should be performed whenever a dataset will be generated on more than a single instrument. When determining the best method for standardization, logistical considerations, as well as the impact on the data, should be considered. Knowing the performance of each instrument is key as well as using the good approach.

ACKNOWLEDGMENT

We want to thank Alan Stall and Aaron J. Tyznik from BD for training, guidance, and lots of fun discussions!

CAPRION.COM