Implementation of Cytek® Aurora Instruments in Clinical Trials: A Multi-step Process Including Performance Qualification and Standardization

Veronica Nash[1], Jan Spitaels[2], Shawn Mehrzad[1], Amber Baele[1], Jarne Schelpe[2], Silke De Waele[2], Miet De Baere[2], Amay Dankar[3], Charlotte Rentenaar[3], Sebastiaan Van Bockstael[3], Nithianandan Selliah[1]

[1]Cerba Research, Ghent, Belgium and New York, USA; [2]Cerba Healthcare Belgium, Division CRI, Ghent, Belgium; [3]Cytek Biosciences, California, USA

Introduction

Flow cytometry plays an important role for patient immune profiling in global clinical studies. Instrument standardization is critical to obtain transparent results across different instruments located in different labs. High parameter assay with deeper characterization of patients' immune subsets in clinical trials utilizing spectral flow cytometer requires developing new methods for instrument implementation and standardization. As there are no specific guidelines on performance qualification (PQ) and instrument standardization for spectral flow cytometers , an in-house workflow was developed for this purpose.

Performance qualification

Standardization

Performance Qualification and Standardization of Cytek® Aurora Instruments: Unleash the Potential of High-Parameter Spectral Flow Cytometry for Global Clinical Trials.

Laser And Detector Performance

During Performance Qualification process, the instrument's optical alignment and system resolution were assessed by daily running of SpectroFlo® QC beads (Cytek® Biosciences) in the software's QC module. Stability of the lasers and detectors was assessed by daily acquisition of the SpectroFlo® QC beads in a user-defined acquisition module using default Cytek assay setting (CAS). Deviations of the daily median fluorescence intensity (MFI) readings were calculated against MFI target values established at installation and expressed as %difference. MFI deviation was calculated for all 64 detectors (as a representative data, only yellow-green laser is shown in Figure 1A). All the data show % difference within our acceptance criteria of <5%.

In addition, detector linearity was determined. SPHERO™ UltraRainbow calibration beads (Spherotech) were measured daily for ten subsequential days using default CAS. MFI values for all detectors were converted into molecules of equivalent fluorochrome (MEF) values and plotted into a regression line (MEF vs relative channel, values of beads provided by Spherotech). Slope, intercept and R^2 values were extrapolated and were within CLSI H62 acceptance criteria for each detector ($R^2 \cong 1$) indicating the linearity of the detectors over time. Figure 1B shows R^2 values over time (up to 10 days) for Yellow-green detector, as a representative data. Data for all other detectors are similar.

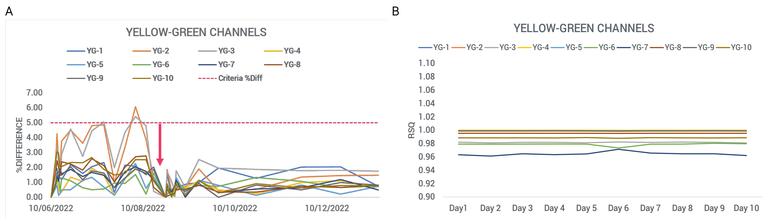


Figure 1: (A) Deviations of the daily median fluorescence intensity (MFI) readings over time for yellow-green detectors. %difference should be below 5% (dotted pink line). Arrows indicate when optics were realigned. **(B) Detector linearity (R^2 values) over time.** R^2 should be ≥ 1

MFI Target Values Alignment

MFI target values, obtained by acquiring the same lot of SpectroFlo® QC beads with CAS in an acquisition module were compared to assess standardization between two Cytek® Aurora instruments in different locations (Figure 2). All detectors have %CV and %difference within the acceptance criteria of 5% (majority are even below 2-3%) which indicates that the two instruments are comparable.

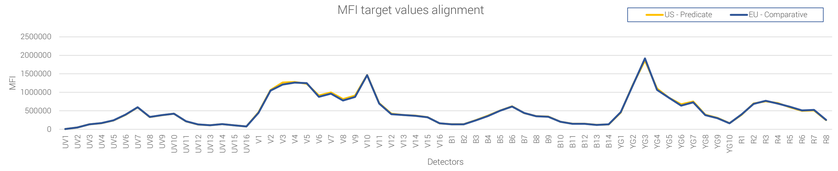


Figure 2: MFI per detector (64 in total) for predicate, US, and comparative, EU instruments. Lines are almost indistinguishable as the variation is very low.

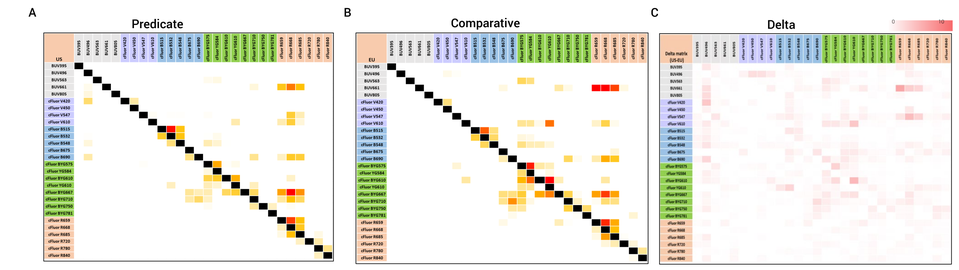
Instrument Characterization

Cryopreserved peripheral blood mononuclear cells (PBMC) were single stained with anti-CD4 antibodies conjugated to 29 different fluorochromes whose emission span the full emission spectrum. CAS was used for acquisition. MFI output for each fluorochrome was recorded and a spillover spread matrix (SSM) was created for both predicate and comparative instrument (Figure 3, A and B) . The resulting matrix is unique to each instrument and can be used as quality control and benchmarking tool to monitor instrument performance and support panel design.

A Valuable Tool For Instrument Comparison

SSM matrices for predicate and comparative instrument show a similar pattern, when compared side by side (Figure 3, A and B). To assess instrument comparison, the delta matrix between the two SSMs was calculated (Figure 3C). Values are within a delta of 10 which is in line with Cytek's observations for instrument comparison.

Figure 3: Heatmaps based on the SSM. (A) Predicate instrument, US. **(B)** Comparative instrument, EU. **(C)** Delta matrix of Predicate and Comparative instrument.



Conclusion

The results shown here demonstrate that:

1. The instruments are comparable as variation of the MFI target values was between 2-5 %CV and the SSM delta matrix values, calculated between the SSM obtained from each instrument, are within Cytek's specifications for instrument comparison.
2. The performance between the two instruments was consistent as MFI output and populations frequencies obtained from immunophenotyping assay showed ≤ 20% difference.

The standardization methods described above provide guidance on how to implement Cytek® Aurora instruments to generate transparent results for flow cytometry assays run in global clinical trials.

Assay Performance Comparison On Biological Relevant Assay

To assess comparability between instruments, an 18-color pre-stained lyophilized PBMC kit (same lot number and reconstitution protocol) was used. MFI and population frequencies were used as a measure of assay performance between the instruments.
%difference between population frequency (Figure 4A and Table 1) and MFI (Figure 4B and Table 2) obtained from the two instruments is below our acceptance criteria of 20%, except for rare populations (with frequency below 5%), where a higher statistical variability is expected.

Figure 4: Results for assay performance comparison between predicate Instrument (US) and comparative instrument (EU) on 18-color pre-stained kit of lyophilized PBMC. **(A)** Population frequency comparison for different biological relevant populations. **(B)** Overlay of the MFI values for different markers.

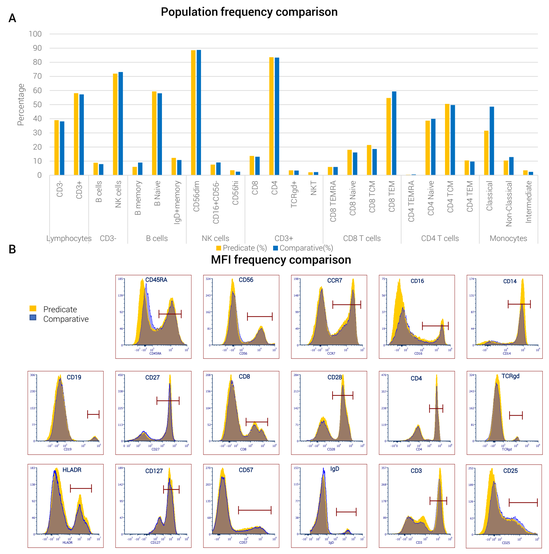


Table 1: %difference between predicate and comparative instrument for different population frequencies on 18-color pre-stained kit of lyophilized PBMC.

Parental population	Reportables	%difference
Lymphocytes	CD3-	1.50
	CD3+	1.01
CD3-	B cells	7.58
	NK cells	1.09
B cells	B memory	**28.90***
	B Naive	1.51
	IgD+memory	8.67
NK cells	CD56dim	0.18
	CD16+CD56-	12.59
	CD56hi	**20.95****
CD3+	CD8	2.72
	CD4	0.22
	TCRgd+	2.31
	NKT	4.95
CD8 T cells	CD8 TEMRA	0.00
	CD8 Naive	7.21
	CD8 TCM	9.10
	CD8 TEM	5.55
CD4 T cells	CD4 TEMRA	**36.76****
	CD4 Naive	2.20
	CD4 TCM	1.00
	CD4 TEM	5.22
Monocytes	Classical	**30.43****
	Non-Classical	14.56
	Intermediate	**23.33****

Values in bold exceed the criteria of 20% difference. (*) event count close to 100. (**) population frequency below 5%. (***) higher difference possible due to monocyte variability in PBMCs

Table 2: %difference between predicate and comparative instrument for different MFI frequencies on 18-color Pre-stained kit of lyophilized PBMC.

Marker	Fluorochrome	%difference
CD25	PE	3.84
CD45RA	BUV395	0.28
CD56	BUV737	1.15
CCR7	BV421	0.20
CD16	eFluor 450	0.33
CD14	BV510	8.32
CD8	BV570	2.94
CD4	BV650	0.19
CD19	BV711	1.38
CD28	BV785	1.11
CD3	Alexa Fluor 488	11.18
TCRgd	PerCP-eFluor 710	2.94
IgD	PE/Dazzle594	5.17
CD57	PE-Cy7	4.96
CD27	APC	1.52
CD127	APC-R700	1.46
HLADR	APC/Fire 750	6.08

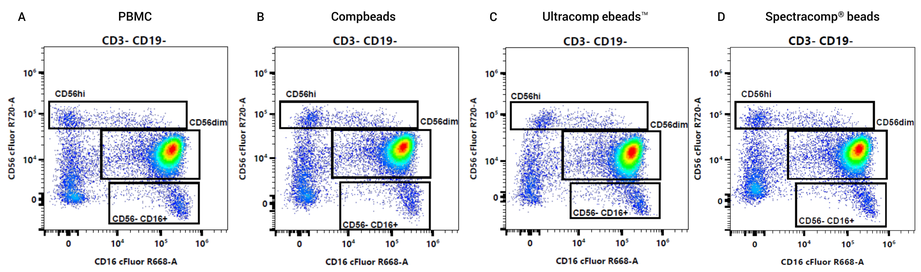
$$\%difference = ABS\left(\frac{Mean\ Instrument\ 1 - Mean\ Instrument\ 2}{Grand\ Mean\ of\ (\ Mean\ Instrument\ 1\ and\ \ Mean\ Instrument\ 2)} \times 100\right)$$

Analytical Performance – Unmixing With Cells Versus Beads

Analytical validation of the instrument was performed on PBMCs from 3 different donors using Cytek® cFluor™ IP Kit 14 Color (Cytek® Biosciences) for immunophenotyping of T, B, NK cells and monocytes.

During assay setup, different options for reference controls were evaluated with PBMCs (Figure 5). Error-free unmixing was achieved when using PBMCs or SpectraComp® beads. Testing of SpectraComp® beads as reference controls is suggested for spectral unmixing, to reduce errors due to PBMCs batch-to-batch variability and optimize workflow.

Figure 5: Spectral unmixing comparison between PBMCs and beads for use as reference controls. (A) PBMC **(B)** BD CompBeads **(C)** Thermofisher UltraComp eBeads™ **(D)** SlingShot SpectraComp® beads



Assay Performance Correlation In Two Instruments With The Assay Of Interest

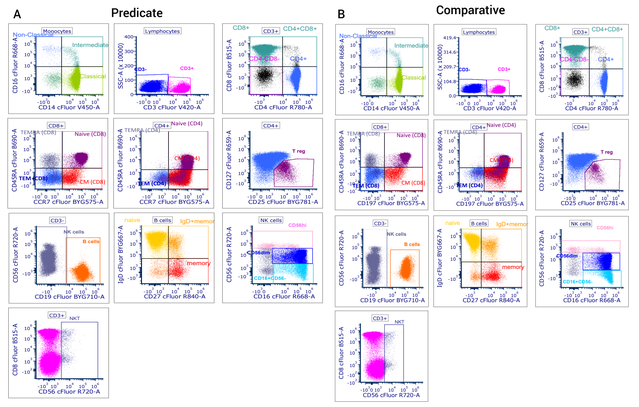
The same lot of immunophenotyping Cytek® kit (Cytek® cFluor™ IP Kit 14 Color) was used on the predicate and comparative instruments with the same 3 PBMC donors to assess instrument standardization. Data show that both instruments provide visually similar profiles (Figure 6). In addition, it shows that the performance between the two instruments is consistent as the obtained population frequencies shown are below 20% difference, except for rare populations (with frequency below 5%) (Table 3).

Table 3: %difference between predicate and comparative instrument for different reportables over three donors stained with Cytek® cFluor™ IP Kit 14 Color .

Parental Population	Reportables	%difference		
		Donor 1	Donor 2	Donor 3
% Leuko	Lymphocytes	1.32	0.26	1.48
% Ly	CD3-	1.67	2.59	9.82
	T Cells	0.10	0.65	1.25
% T	CD4+ T Cells	2.25	2.41	5.89
% CD4+ T	Naive	1.85	0.39	2.86
	CM	3.49	3.21	3.03
	TEM	0.69	12.84	3.29
	TEMRA	**54.87***	11.02	**26.87***
	T regulatory cells	5.42	9.25	4.08
% T	CD8+ T Cells	1.21	1.98	8.94
% CD8+ T	Naive	0.13	2.98	5.68
	CM	12.95	12.82	6.64
	TEM	5.57	11.66	8.92
	TEMRA	4.20	5.65	12.14
% Ly	NKT cells	8.11	5.06	2.93
% Leuko	Monocytes	1.36	12.55	17.26
% Mono	Classical	2.56	0.05	2.99
	Intermediate	**41.93***	16.28	4.44
	Non-classical	**46.24***	**71.33***	**34.63***
% Ly	B cells	9.61	9.52	13.98
% B	Naive	1.82	1.19	0.29
	Memory	15.23	11.35	8.85
	IgD+ memory	9.93	15.53	2.27
% Ly	NK cells	9.34	3.02	2.81
% NK	CD56hi	0.15	15.84	10.36
	CD56dim	10.10	3.07	3.21
	CD56-CD16+	5.20	19.07	**20.47***

Values in bold exceed the criteria of 20% difference. (*) population frequency below 5%.

Figure 6: Gating strategy for Cytek® cFluor™ IP Kit 14 Color **(A)** Predicate Instrument, US. **(B)** Comparative Instrument, EU.



Cerba Research
www.cerbaresearch.com
Flow Cytometry Science Team
flowcytometry@cerbaresearch.com

Cerba Research can develop and validate customized flow cytometry panels for global clinical trials. Connect with our scientific team to learn how we can enhance your research and develop specific flow cytometry panels.

For more information please contact
Veronica Nash, US Regional Head of Flow Cytometry
vnash@cerbaresearch.com