Cytek Aurora Instrument Standardization for Patient Testing in Global Clinical Trials

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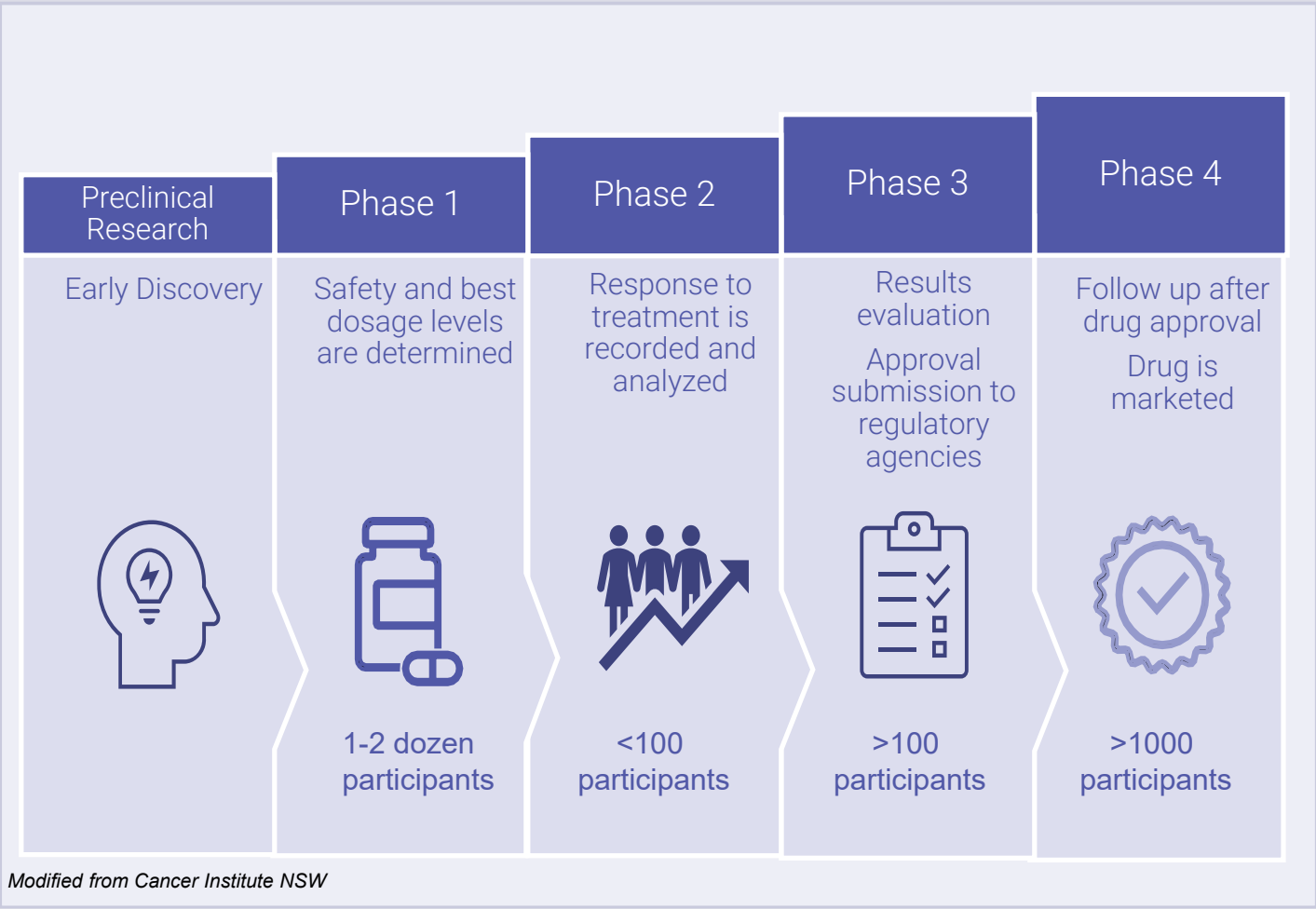
Cytek UGM, Amsterdam, June 20th, 2023

Presentation Overview

- Introduction:
  - Use of Flow Cytometry in different Phases of Clinical Trials
- Cytek Aurora Implementation in Clinical Labs for Multi-Site Studies:
  - Installation Qualification/Operational Qualification
  - Performance Qualification – Analytical Performance
  - Instruments Standardization – Performance Comparison between Instruments
- Conclusions
- Q&A - discussion

Flow Cytometry Supports All Phases of Clinical Trials



Modified from Cancer Institute NSW

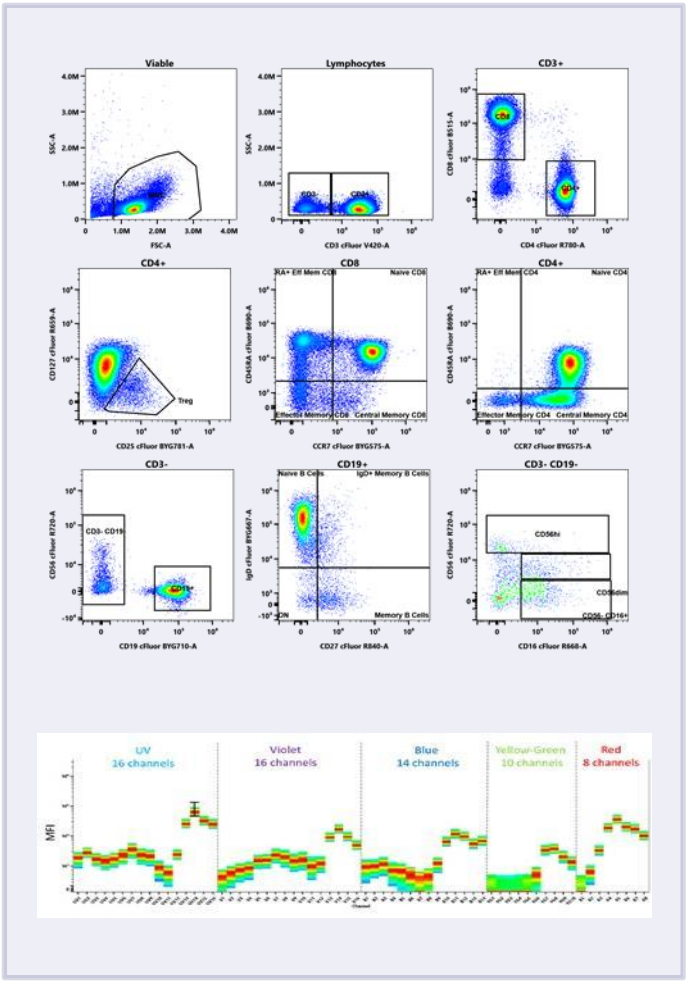
- Flow Cytometry Assays
  - Immune cells enumeration and characterization
    - ✓ TBNK
    - ✓ T cells, monocytes, NK cells, B cell subsets
  - Monitoring and characterization of therapeutic cells
    - ✓ CAR T cells
  - Cellular status characterization
    - ✓ Cell activation, exhaustion, etc.
  - Detection of intracellular cytokines (ICS)
  - Drug kinetic
    - ✓ Receptor occupancy assays
  - Immunophenotyping for detection and monitoring of biomarkers

Multi-location Studies Require Method and Instrument Standardization in Different Labs

- Standardization
  - Same results are expected independently from where the assay is performed and who performs it
- Achieved by
  - Instrument platform
  - Validation process
  - Assay transfer
  - Protocol/process (SOP)
  - Centralized data analysis and review

Cytek Aurora: Power of High Dimensional Flow in Clinical Trials

- Deep cell characterization and immune-monitoring:
  - Fulfill exploratory needs for new biomarkers identification
- Multiple cells characterization from the same tube
  - Data from samples with limited availability (BMA)
- High data quality

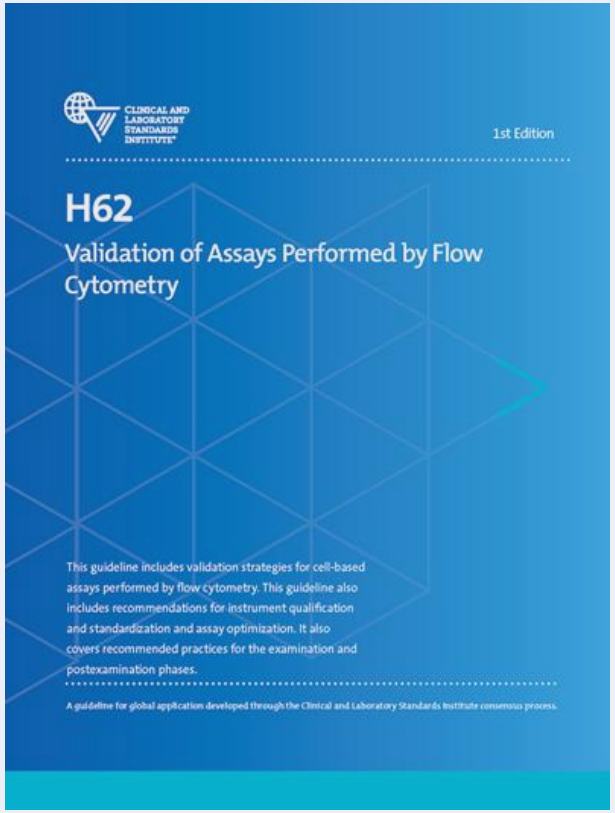


Implementation and standardization of

Cytek Aurora in our US and EU labs

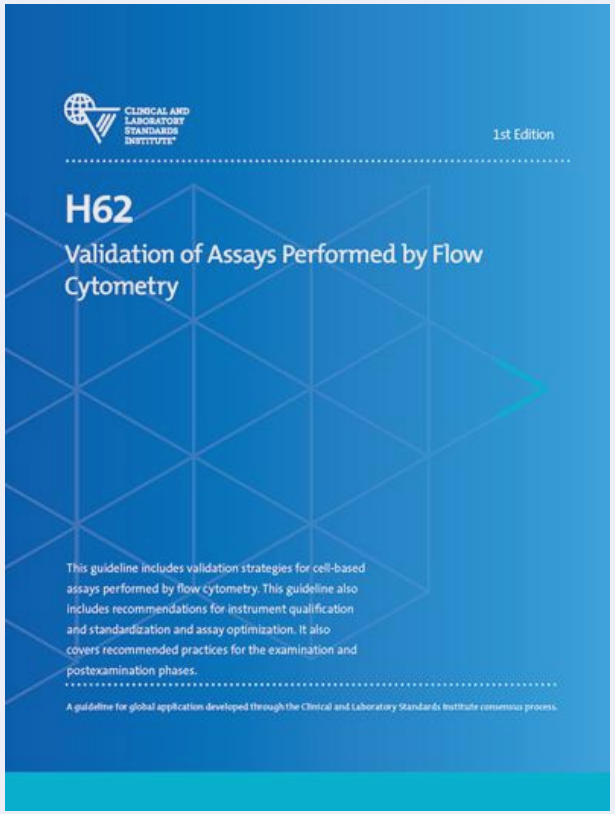
Standardization of Cytek Aurora Instruments is a Multi-Step Process

- Instrument Installation
- Performance Qualification
- Instrument Standardization



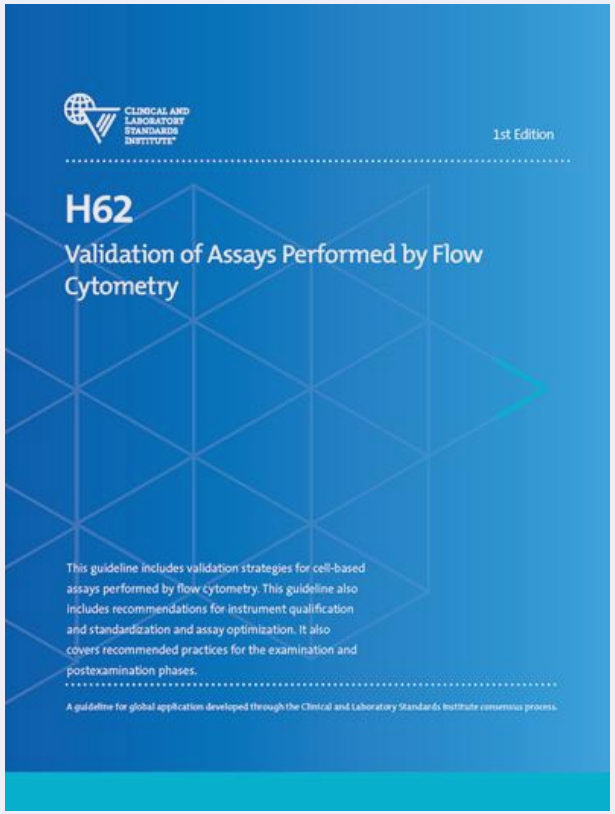
Standardization of Cytek Aurora Instruments is a Multi-Step Process

- Instrument Installation
  - Installation Qualification/Operational Qualification (IQ/OQ) by Field Service Engineer (FSE)

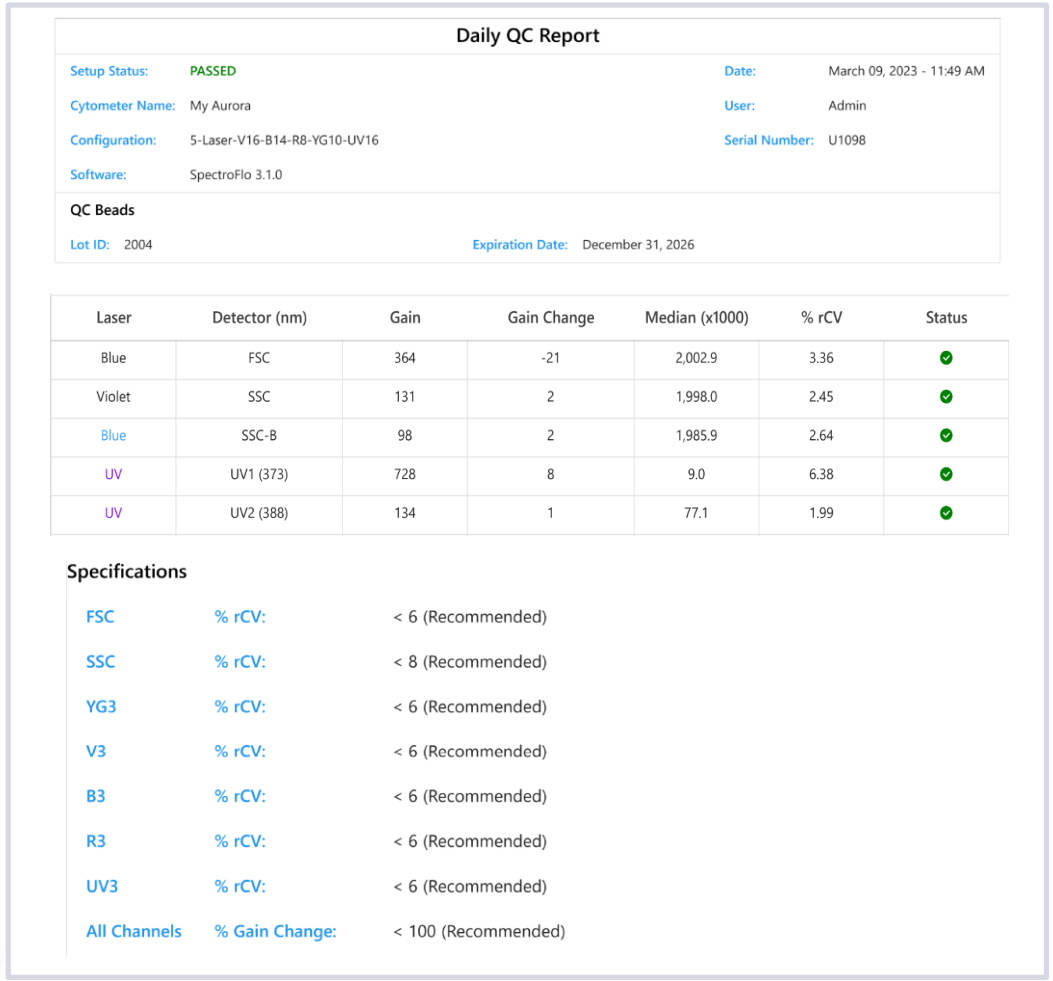


Standardization of Cytek Aurora Instruments is a Multi-Step Process

- Instrument Installation
  - ✓ IQ/OQ (FSE)
- Performance Qualification
  - Laser and Detectors Performance
  - Detectors Stability
  - Analytical Performance



Laser and Detectors Performance is Assessed and Monitored Daily with QC



Daily QC Report

Setup Status:	PASSED	Date:	March 09, 2023 - 11:49 AM
Cytometer Name:	My Aurora	User:	Admin
Configuration:	5-Laser-V16-B14-R8-YG10-UV16	Serial Number:	U1098
Software:	SpectroFlo 3.1.0		

QC Beads

Lot ID: 2004 Expiration Date: December 31, 2026

Laser	Detector (nm)	Gain	Gain Change	Median (x1000)	% rCV	Status
Blue	FSC	364	-21	2,002.9	3.36	✓
Violet	SSC	131	2	1,998.0	2.45	✓
Blue	SSC-B	98	2	1,985.9	2.64	✓
UV	UV1 (373)	728	8	9.0	6.38	✓
UV	UV2 (388)	134	1	77.1	1.99	✓

Specifications

FSC	% rCV:	< 6 (Recommended)
SSC	% rCV:	< 8 (Recommended)
YG3	% rCV:	< 6 (Recommended)
V3	% rCV:	< 6 (Recommended)
B3	% rCV:	< 6 (Recommended)
R3	% rCV:	< 6 (Recommended)
UV3	% rCV:	< 6 (Recommended)
All Channels	% Gain Change:	< 100 (Recommended)

Run daily on the instrument with SpectroFlo® QC beads to check for optical alignment, detectors linearity and dynamic range, and overall resolution of the detection system

QC must be done daily before use and PASS

Laser and Detectors Performance is Monitored Daily with SpectroFlo® QC Beads

- Determination of baseline MFI target values with CytekAssaySetting (CAS) using SpectroFlo® QC beads in Acquisition Module
- Beads are run daily, after QC and variation from baseline is calculated (%difference)
- Our variation acceptance criteria is 5% difference

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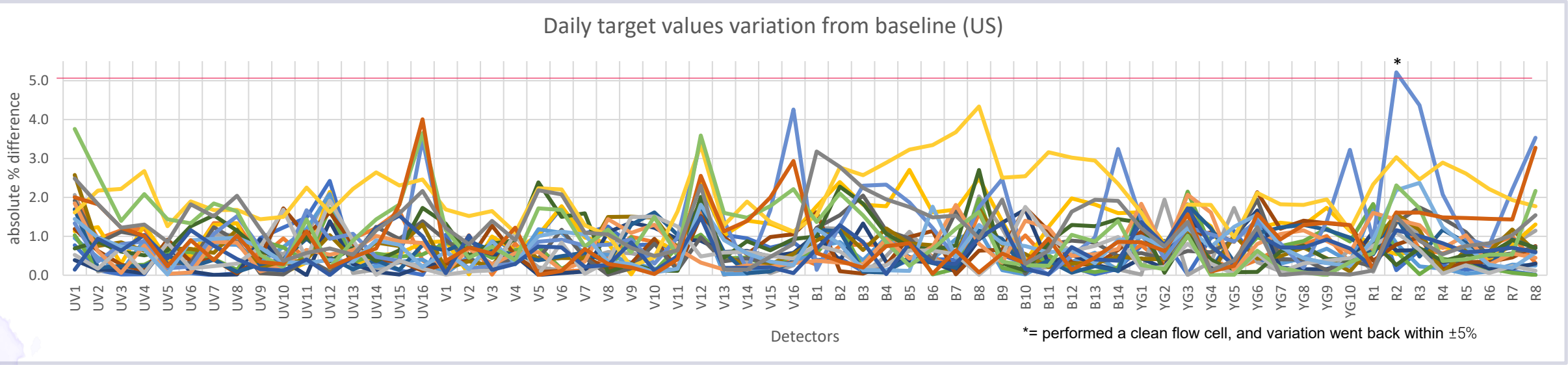


Over time, instrument demonstrates low variation in MFI values in all detectors

Assay Performance Correlation in Two Instruments with the Assay of Interest

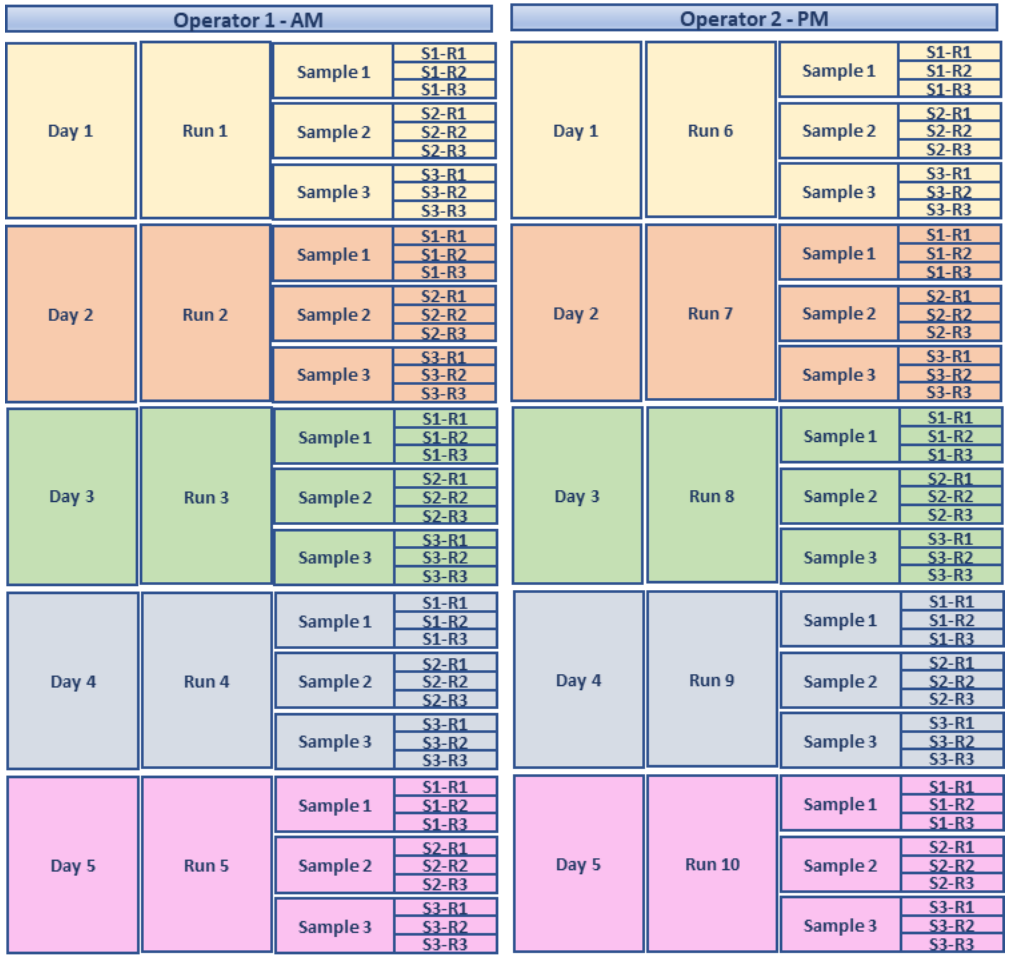
- TBNKM_CR14C assay
  - Cytek cFluor™ IP Kit 14 Color RUO kit for characterization of monocytes, T, B lymphocytes, NK cells and subsets
  - Matrix: cryopreserved PBMC (batch analysis)
  - Reference controls (SpectraComp and ViaComp beads (Slingshot Biosciences))
  - Viadye titration
  - Troubleshooting unmixing errors

Violet		Blue		Red	
Specificity	Fluorochrome	Specificity	Fluorochrome	Specificity	Fluorochrome
CD3	cFluor V420	CD8	cFluor B515	CD127	cFluor R659
CD14	cFluor V450	CCR7	cFluor BYG575	CD16	cFluor R668
CD45	cFluor V547	IgD	cFluor BYG667	CD56	cFluor R720
		CD45RA	cFluor B690	CD4	cFluor R780
		CD19	cFluor BYG710	Viability	ViaDye Red
		CD25	cFluor BYG781	CD27	cFluor R480

- T helper, T cytotoxic, Treg, NKT memory profile (TEMRA, Naïve, CM, EM)
- B cells (Naïve, memory)
- Monocytes (intermediate, classical, non-classical
- NK cells

Analytical Performance Evaluates Whether the Instrument Performs for its Intended Purpose

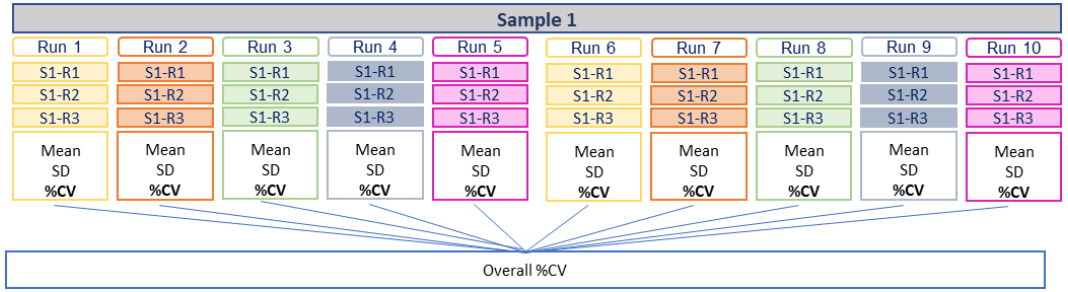
- Assay Validation provides a means to ensure that data are credible and reproducible*:
  - Precision (Repeatability, Robustness)
  - Stability (frozen sample stability, processed sample stability)



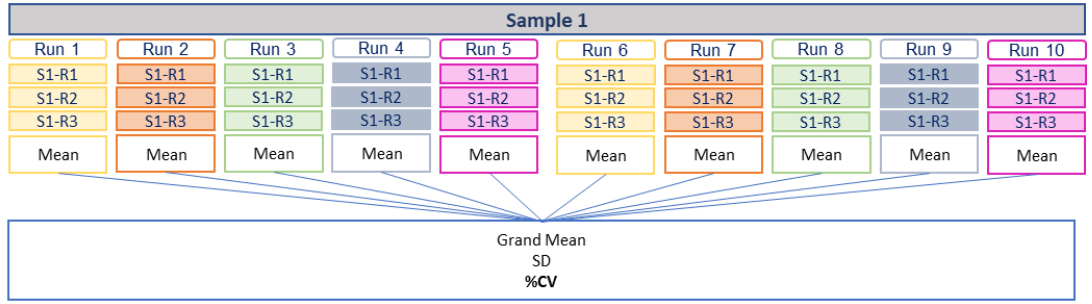
Selliah N., et al, Flow Cytometry Method Validation Protocols, 2019 (new updated version in line with CLSI H62 coming soon)

Analytical Performance Acceptance Criteria and Results

Repeatability: overall %CV ≤ 25



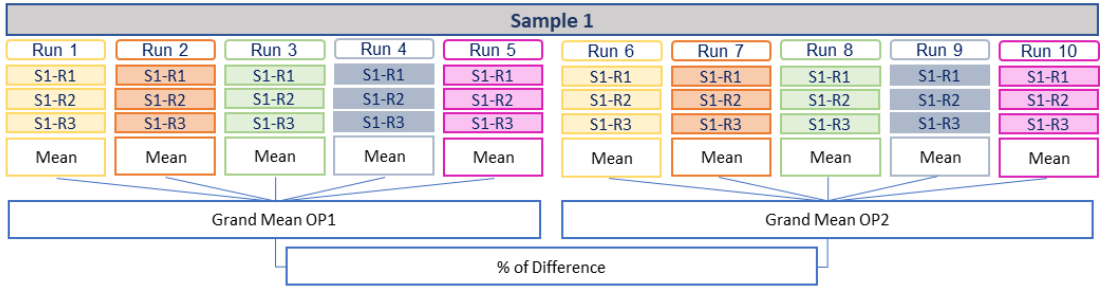
Intermediate precision %CV ≤ 25



Populations with frequency above 5%* are within acceptance criteria

**populations with less than 100 events and frequency below 5% are considered rare populations (acceptance criteria are less stringent)*

Inter-operator variability %difference ≤ 20



Standardization of Cytek Aurora Instruments is a Multi-Step Process

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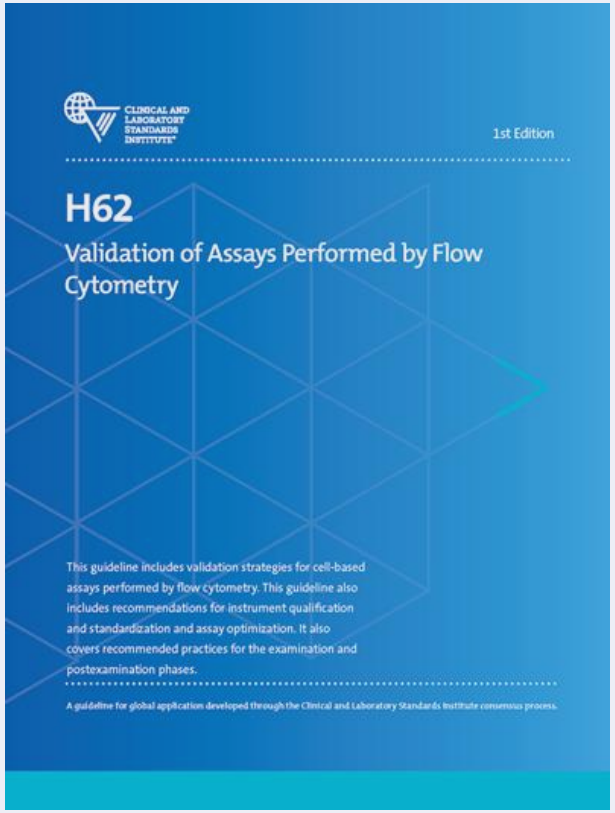

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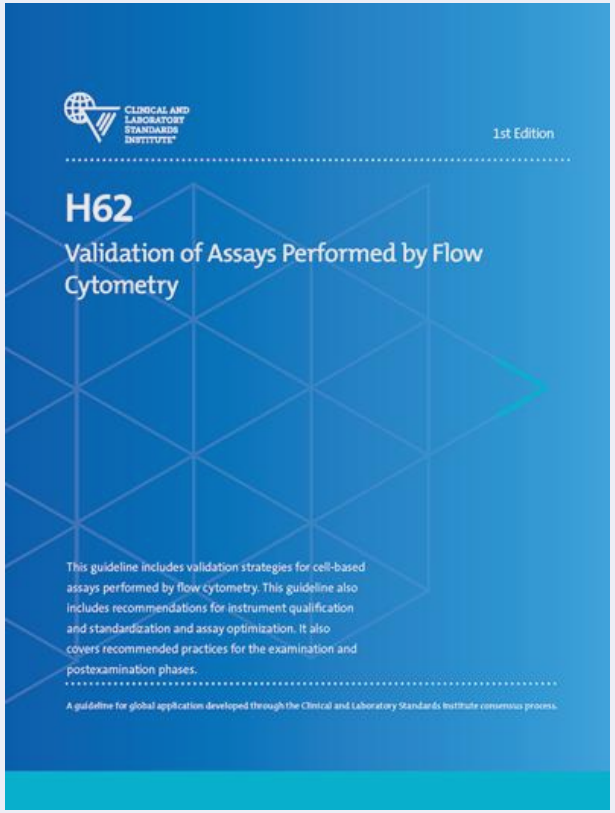
- Instrument Standardization
  - MFI target values variation
  - Comparison of Spillover Spread Matrix (SSM)
  - Assay Performance on 18-color pre-stained kit of lyophilized PBMC



MFI Target Values Alignment Between Instruments

- Definition of predicate and comparative instruments
- Determination of baseline MFI target values at CAS (Cytek Assay Settings) for predicate instrument (US) with SpectroFlo® QC beads in Acquisition Module
- SpectroFlo® QC beads (same lot) are run in Acquisition Module and variation between instruments is calculated
- Alignment of MFI values for comparative instrument (EU) with the ones of predicate instrument (US)

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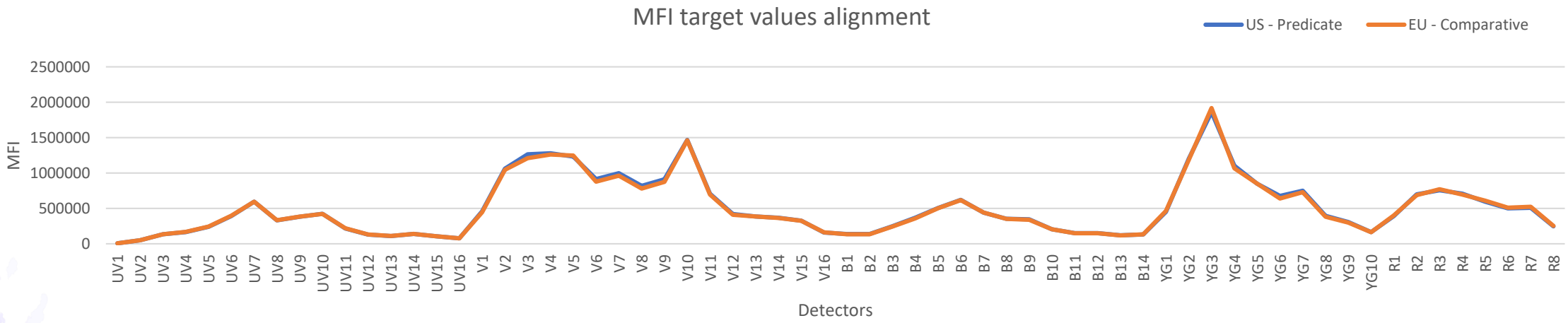
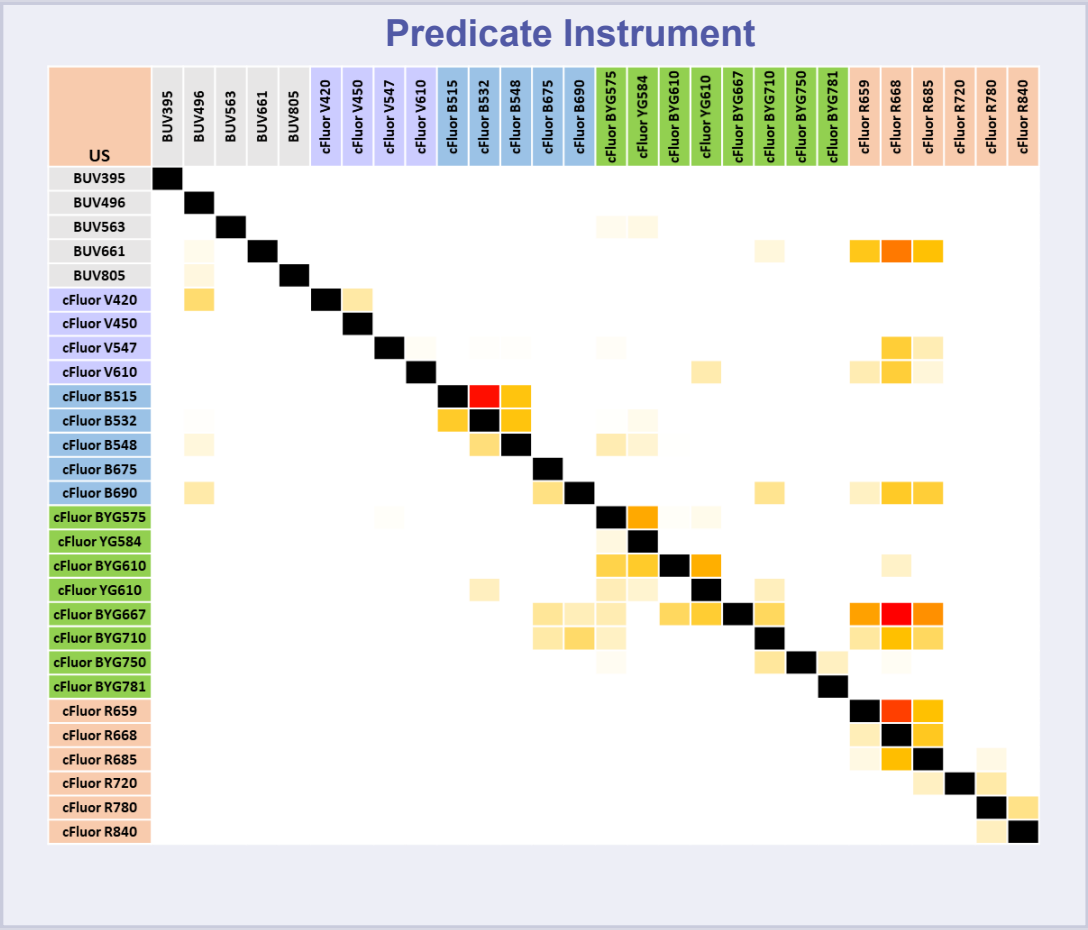
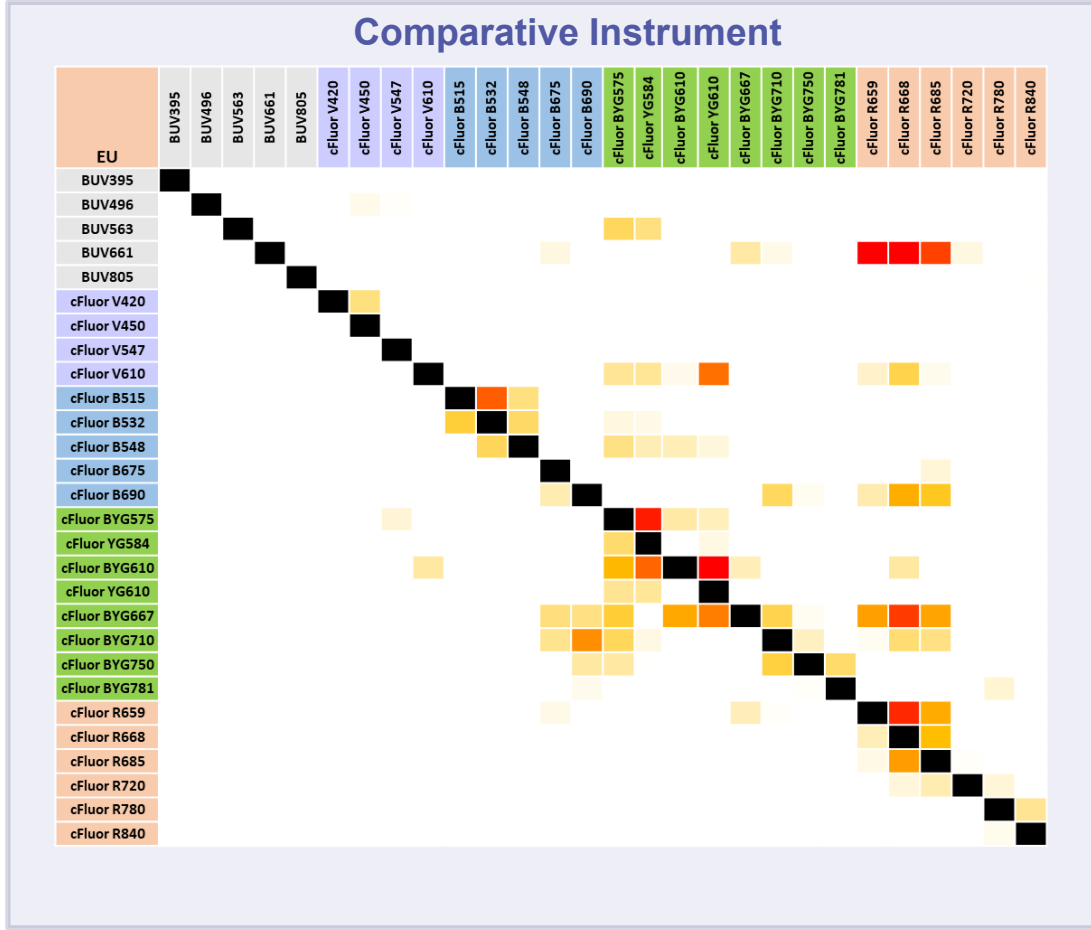
- SpectroFlo® QC beads (same lot) are run in Acquisition Module and variation between instruments is calculated
- Alignment of MFI values for comparative instrument (EU) with the ones of predicate instrument (US)
- **All detectors have %CV and % difference within 5% (majority are below 2-3%)**



Instrument Characterization is Valuable for Instrument Comparison





Delta SSM is calculated, and all values are within a delta of 10

→ in line with Cytek's observations of instrument comparison

Assay Performance Comparison on 18-color Pre-Stained Kit of Lyophilized PBMC

- Use of a biological relevant assay that includes various population frequencies and marker levels of expression
- Advantages
  - Same biological sample and staining (use same kit lot number, same reconstitution protocol)
  - Stable over time
  - Reduces sample process variability and isolate differences due to instrument performance

	UV			Violet			Blue			Yellow/Green			Red		
Emission	355			405			488			561			640		
	Marker	Rec. Dil.	Fluor	Marker	Rec. Dil.	Fluor	Marker	Rec. Dil.	Fluor	Marker	Rec. Dil.	Fluor	Marker	Rec. Dil.	Fluor
395	CD45RA		BUV395												
420				CCR7		BV421									
440															
450	Viability		LIVE DEAD Blue	CD16		eFluor 450									
480															
500				CD14		BV510									
520							CD3		Alexa Fluor 488						
550															
570				CD8		BV570				CD25		PE			
580															
600										IgD		PE/Dazzle 594			
660				CD4		BV650							CD27		APC
680															
690															
700				CD19		BV711	TCR γδ		PerCP-eFluor 710				CD127		APC-R700
730	CD56		BUV737												
750															
780				CD28		BV785				CD57		PE-Cy7			
800													HLA-DR		APC/Fire 810

- T helper, T cytotoxic, Tgd, Treg, NKT, memory profile (TEMRA, Naïve, CM, EM)
- B cells (Naïve, memory)
- Monocytes (intermediate, classical, non-classical
- NK cells

Comparison between Predicate and Comparative Instrument (Statistic)

	Predicate (MFI)	Comparative (MFI)	%Difference
CD25 PE-A	6576	6963	3.84
CD45RA BUV395-A	8688	8725	0.28
CD56 BUV737-A	9808	9639	1.15
CCR7 BV421-A	15218	15173	0.20
CD16 eFluor 450-A	158191	158973	0.33
CD14 BV510-A	75434	66391	8.32
CD8 BV570-A	23253	22242	2.94
CD4 BV650-A	45744	45876	0.19
CD19 BV711-A	43981	43074	1.38
CD28 BV785-A	21910	21546	1.11
CD3 Alexa Fluor 488-A	123070	103519	11.18
TCRGD PerCP-eFluor 710-A	10545	11018	2.94
IGD PE-Dazzle594-A	172926	186692	5.17
CD57 PE-Cy7-A	45284	48742	4.96
CD27 APC-A	34164	33392	1.52
CD127 APC-R700-A	6266	6129	1.46
HLADR APC-Fire 750-A	101246	92290	6.08

- MFI from comparative instrument are compared to the ones from predicate instrument

$$\text{ABS}\left(\frac{\text{Mean Predicate} - \text{Mean Comparative}}{\text{Grand mean of instruments}} \times 100\right)$$

- %difference <20 is considered acceptable

MFI are all within acceptance criteria

Comparison between Predicate and Comparative Instrument (Statistic)

	Population	Predicate (%)	Comparative (%)	%difference
Lymphocytes	CD3-	39.01	38.14	1.50
	CD3+	58.16	57.28	1.01
CD3-	B cells	8.85	7.88	7.58
	NK cells	71.96	73.14	1.09
B cells	B memory	5.98	9.01	28.90**
	B Naive	59.35	58.02	1.51
	IgD+memory	12.35	10.81	8.67
NK cells	CD56dim	88.52	88.76	0.18
	CD16+CD56-	7.54	9.06	12.59
	CD56hi	3.55	2.54	20.95*
CD3+	CD8	13.67	13.12	2.72
	CD4	83.59	83.32	0.22
	TCRgd+	3.5	3.38	2.31
	NKT	2.1	2.26	4.95
CD8 T cells	CD8 TEMRA	5.87	5.87	0.00
	CD8 Naive	18	16.12	7.21
	CD8 TCM	21.44	18.64	9.10
	CD8 TEM	54.69	59.37	5.55
CD4 T cells	CD4 TEMRA	0.37	0.62	36.76*
	CD4 Naive	38.64	39.93	2.20
	CD4 TCM	50.5	49.75	1.00
	CD4 TEM	10.49	9.69	5.22
Monocytes	Classical	31.58	48.58	30.43***
	Non-Classical	10.44	12.9	14.56
	Intermediate	3.51	2.41	23.33*

- Population frequencies from comparative instrument are compared to the ones from predicate instrument
- %difference is calculated as:

$$\text{ABS}\left(\frac{\text{Mean Predicate} - \text{Mean Comparative}}{\text{Grand mean of instruments}} \times 100\right)$$

- %difference <20 is considered acceptable

Populations are within the acceptance criteria, except for rare populations

*population frequency below 5%
**event count close to 100
***higher difference possible due to monocyte variability in PBMC

Standardization of Cytek Aurora Instruments is a Multi-Step Process

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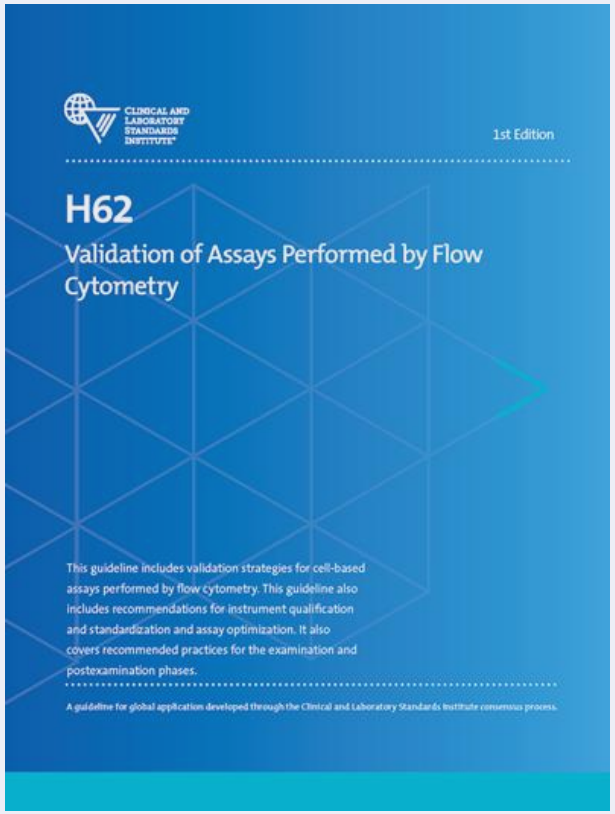

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  - Matrix: cryopreserved PBMC (batch analysis)
  - Reference controls (SpectraComp and ViaComp beads (Slingshot Biosciences))
  - Assay is validated according to CLSI H62 guidelines (Precision, Inter-Operator, Stability)

Violet		Blue		Red	
Specificity	Fluorochrome	Specificity	Fluorochrome	Specificity	Fluorochrome
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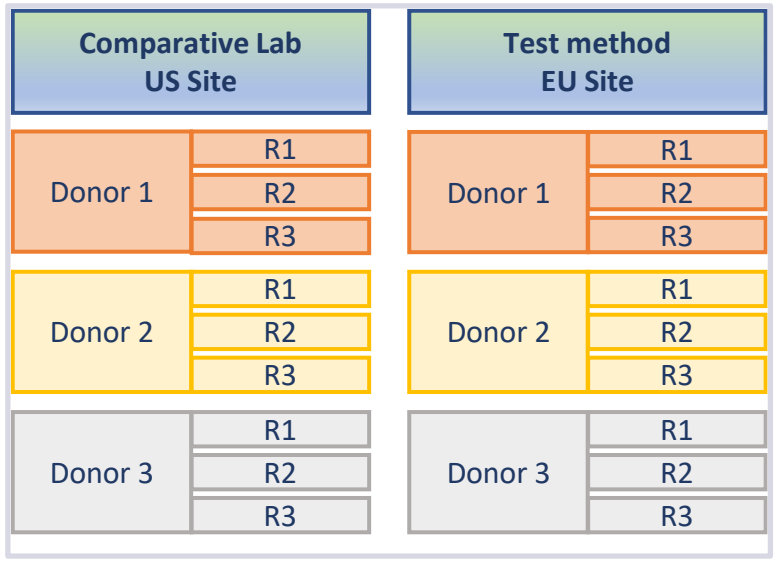
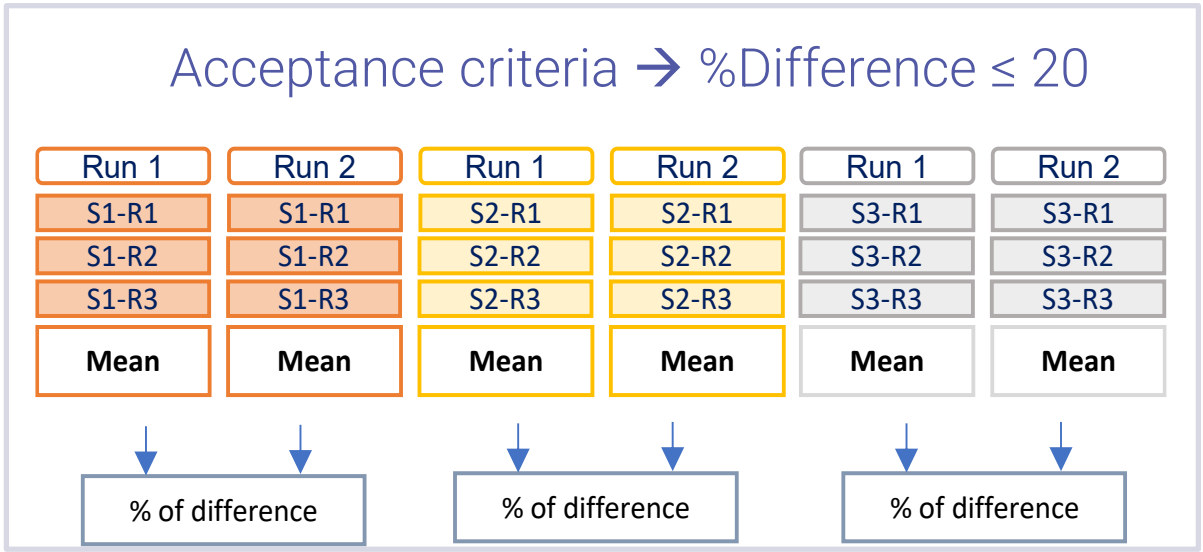
Assay Performance Correlation in Two Instruments with the Assay of Interest

- TBNKM_CR14C assay
  - The comparative lab (US) transfers settings (CAS-1, threshold modification), optimized protocol, analysis template and gating strategy to EU lab
  - Correlation experiment (3 donors, same for both sides): comparison of all populations of interest between instruments





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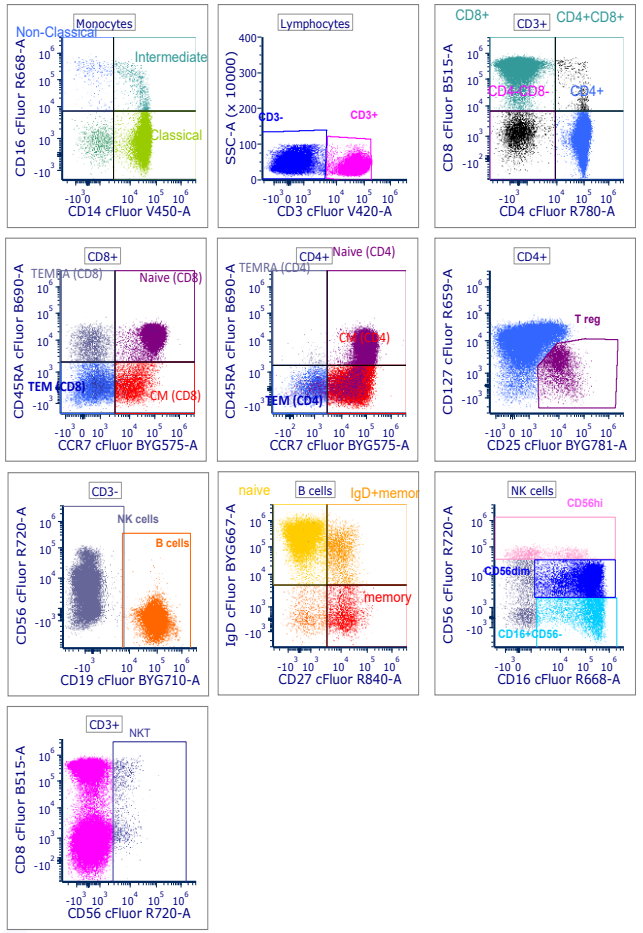
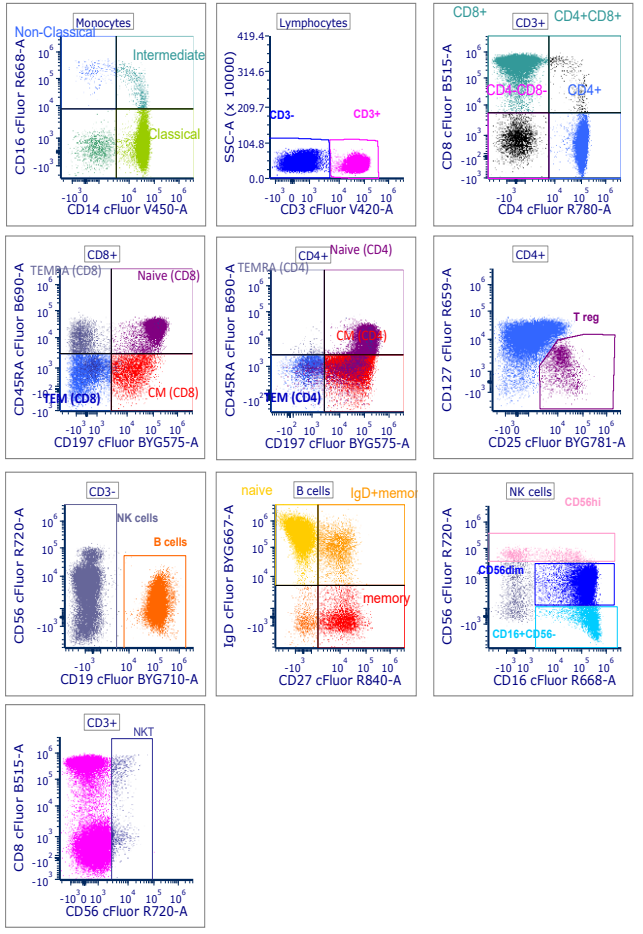
Performance Correlation Passes Acceptance Criteria for All Populations of Interest

Predicate Instrument



Comparative Instrument



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	Naïve	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	Naïve	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	Naïve	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*

*population frequency below 5%

Conclusions

- Reproducibility of data across different time points and between different instruments requires proper standardized assays

- At Cerba Research, standardization for Cytek Aurora Instruments for use in Clinical Trials is achieved and monitored by different methods:
  - MFI target values at CAS variation within 5% (target MFI values monitored daily)
  - Assay performance instrument comparison (% and MFI) with a biological sample
  - SSM comparison
  - Standardization is tested on relevant assay confirming instrument standardization and readiness for clinical trials

- **Our work demonstrates that Cytek Aurora instruments offer optimal standardization and can be reliably used in drug development with the advantage of deeper immune profiling even with limited patient samples**

Acknowledgements

Cerba HealthCare Belgium

- **Miet De Baere**
- Jan Spitaels
- Silke De Waele
- Feyzâ Matisli
- **Jarne Schelpe**

Cerba Research
Your precision medicine partner

- Nithianandan Selliah
- **Veronica Nash**
- Leen Catrysse
- Matthias De Decker
- Bieke Soen
- Christopher Rota
- Sanaz Koosha
- **Shawn Mehrzad**
- **Amber Baele**
- Malcolm Mezue
- Manpreet Singh
- Eva Van Lombergen
- Alice Umulisa
- Annabel Njang Chia

CYTEK
TRANSCEND THE CONVENTIONAL

- **Charlotte Rentenaar**
- **Sebastiaan Van Bockstael**
- Amay Dankar
- Karla Gorena