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A Process for Instrument Performance Qualification Aligned with the CLSI H62 Guideline Using Beads with NIST Assigned ERF Values

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ABSTRACT

- It is essential that all flow cytometers are fully qualified and monitored to ensure that the output generated is reproducible and precise.
- When flow cytometric assays are incorporated into global clinical trials the instrument qualification and monitoring must also include cross-standardization.
- This poster describes an approach for instrument performance qualification (PQ) and cross-standardization of two BD® FACSymphony™A5, Spectrally Enabled, 5-laser (349nm, 405 nm, 488 nm, 561 nm, and 637 nm), 48-color flow cytometers.

Laser Line	Detector	Channel	Mirror (LP)	Filter (BP)	Parameter
UV (349 nm)	A	40	765	809/82	UV809
	B	39	704	736/64	UV736
	C	38	675	695/40	UV695
	D	37	645	660/30	UV660
	E	36	595	610/30	UV610
	F	35	570	585/30	UV585
	G	34	535	540/20	UV540
	H	33	495	515/60	UV515
	I	32	425	446.5/67	UV446
	J	31	365	379/34	UV379
Violet (405 nm)	A	28	810	845/70	V845
	B	27	765	785/50	V785
	C	26	730	750/40	V750
	D	25	690	710/40	V710
	E	24	665	680/30	V680
	F	23	645	660/30	V660
	G	22	605	615/25	V615
	H	21	585	595/30	V595
	I	20	570	576/20	V576
	J	19	530	540/20	V540
	K	18	495	510/40	V510
	L	17	465	470/15	V470
	M	30	430	450/40	V450
	N	29	415	427/25	V427
Blue (488-nm)	A	10	770	810/79	B810
	B	9	724	750/60	B750
	C	8	685	710/50	B710
	D	7	665	675/20	B675
	E	6	645	660/30	B660
	F	5	585	602/40	B602
	G	4	570	576/20	B576
	H	3	520	537/32	B537
	I	2	500	510/20	B510
	J	1	_	488/10	SSC
Yellow-Green (561 nm)	A	49	800 LP	825.5/49 BP	YG825
	B	48	750 LP	780/60 BP	YG780
	C	47	735 LP	750/40 BP	YG750
	D	46	699 LP	730/50 BP	YG730
	E	45	680 LP	695/40 BP	YG695
	F	44	665 LP	670/20 BP	YG670
	G	43	645 LP	660/30 BP	YG660
	H	42	595 LP	602/40 BP	YG602
	I	41	570 LP	585/30 BP	YG585
Red (637 nm)	A	16	750 LP	780/60 BP	R780
	B	15	720 LP	730/50 BP	R730
	C	14	699 LP	710/25 BP	R710
	D	13	680 LP	680/30 BP	R680
	E	12	665 LP	675/20 BP	R675
	F	11	645 LP	660/30 BP	R660

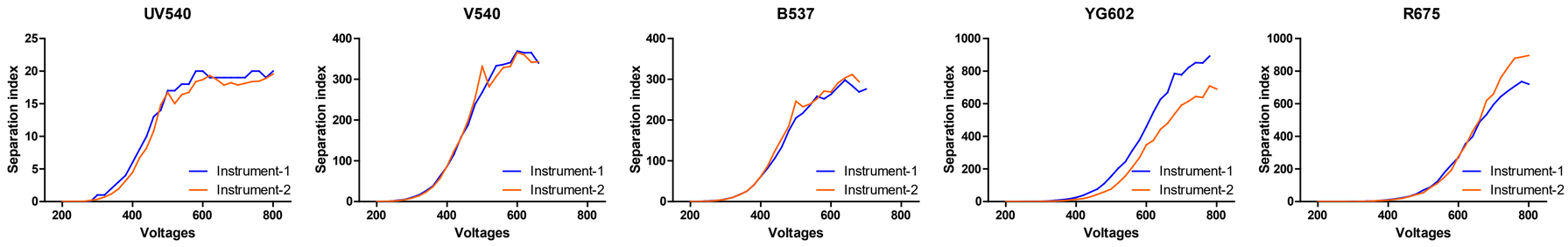
- Instrument qualification (IQ) and operational qualification (OQ) were performed by the vendor field service engineer to verify that the instruments were installed appropriately and performing as intended.
- For the instrument PQ, detector linearity, dynamic range, detector efficiency (Qr), electronic noise (SDen) and background signal (Br) were evaluated for each fluorescence detection channel as described in **CLSI H62**.
- Optimal voltages were established using two approaches- Voltage titration and BD®'s equalizer tool.
- Spherotech Supra Rainbow Quantitative Particles (SRQP) with National Institute of Standards and Technology (NIST) assigned Equivalent Reference Fluorophores (ERF) values were used to determine Linearity and Dynamic Range.
- Detector Linearity assessment methods- Ratiometric and ERF.
- Qr (Detector efficiency) and Br (Background) were obtained from the Cytometer Setup and Tracking (CS&T) Baseline Reports.
- Carryover was evaluated using Streck CD-Chex CD4 Low and CD-Chex Plus Normal quality control (QC) samples.

RESULTS

Establishing Optimal Voltage Settings

Optimal voltages were established using two approaches- 1) Voltage titration and Becton Dickinson's (BD®) equalizer tool (see Poster # 306); 2) Separation index (SI) was calculated from Voltage titration experiments in which the unstained peripheral blood mononuclear cells (PBMC) and BD® CS&T beads were used.

BD®'s equalizer tool was used to adjust voltages for each detector so that the robust standard deviation (rSD) of PBMC was approximately 3X the electronic noise (SDen) derived from the Baseline Report. Optimal voltages were then saved as "Application Settings."

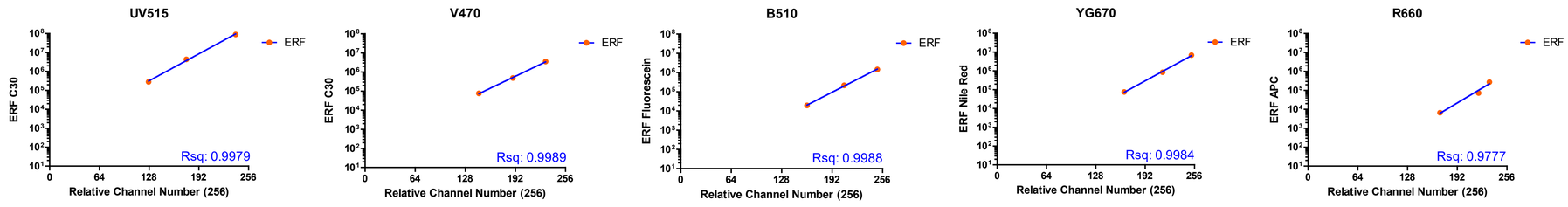


- Voltage titration results in one detector across each laser line.

Linearity Assessment

ERF Approach

- SRQP were acquired at optimal voltages.
- Linearity was confirmed in the detectors where all 4 peaks were on scale. For the remaining detectors, Ratiometric approach was used to confirm linearity.



Bead Population	MdFI	rSD	%rCV	ERF
Peak 1	72	32	44	N/A
Peak 2	496	40	8	281708
Peak 3	5328	203	4	4324709
Peak 4	117098	1918	2	89408032

Bead Population	MdFI	rSD	%rCV	ERF
Peak 1	179	53	30	N/A
Peak 2	1196	74	6	75746
Peak 3	9926	254	3	483589
Peak 4	75918	2847	4	3553180

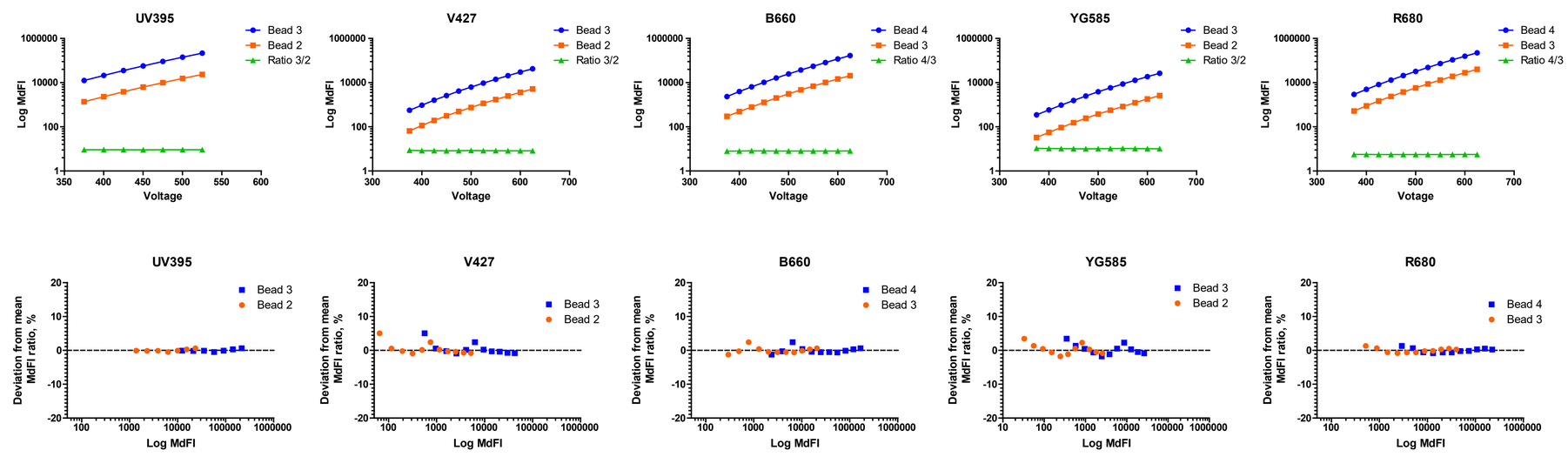
Bead Population	MdFI	rSD	%rCV	ERF
Peak 1	27	25	91	N/A
Peak 2	2463	81	3	19244
Peak 3	24405	506	2	214567
Peak 4	189545	4744	3	1444134

Bead Population	MdFI	rSD	%rCV	ERF
Peak 1	11	48	452	N/A
Peak 2	3098	182	6	75574
Peak 3	35040	893	3	845442
Peak 4	216145	5872	3	6828432

Bead Population	MdFI	rSD	%rCV	ERF
Peak 1	188	56	30	N/A
Peak 2	3938	274	7	6581
Peak 3	44444	1176	3	72418
Peak 4	87228	3428	4	271813

Ratiometric Approach

Representative graphs from one detector across each laser line from one instrument are shown.



- MdFI ratios of the 2 brightest beads are plotted as a function of the PMT voltage to determine the linear range. Representative graphs from one detector across each laser.
- % Difference was calculated between MFI ratios and the mean MFI ratio. Graphs illustrate deviations from linearity across the detector's entire dynamic range.

Detector Efficiency and Background

Parameter	Qr	Br	Parameter	Qr	Br	Parameter	Qr	Br	Parameter	Qr	Br	Parameter	Qr	Br
UV379	2.5813	935	V427	0.3177	387	B510	0.262	222	YG585	0.2942	79	R660	0.14	15
UV446	1.7379	473	V450	0.4041	322	B537	0.9317	260	YG602	0.5414	82	R675	0.1024	0
UV515	0.3138	2177	V470	0.3534	423	B576	0.2479	538	YG660	0.1622	0	R680	0.1393	7
UV540	0.0896	2622	V510	0.6823	66	B602	0.6791	433	YG670	0.0941	114	R710	0.1895	0
UV585	0.04	2021	V540	0.55	84	B660	0.1916	55	YG695	0.1011	600	R730	0.159	0
UV610	0.0351	1644	V576	0.1954	288	B675	0.1483	42	YG730	0.1639	0	R780	0.1863	0
UV660	0.0257	574	V595	0.2635	310	B710	0.1805	24	YG750	0.1482	0			
UV695	0.022	588	V615	0.1633	362	B750	0.1803	0	YG780	0.2056	0			
UV736	0.0693	157	V660	0.1047	243	B810	0.1596	0	YG825	0.1612	0			
UV809	0.0416	96	V680	0.0813	158									
			V710	0.1105	0									
			V750	0.1152	0									
			V785	0.1057	0									
			V845	0.1096	0									

- Qr and Br values provided information on instrument sensitivity.
- Values from one instrument are shown

Carryover

BD® FACS Symphony A5 SE	% Carryover	Manufacturer's Specifications	Mean %CV	Mean Inter-instrument % CV
Instrument 1	-1.58%	<0.75%	2.4	2.2
Instrument 2	0.05%	<0.75%	1.7	

- CD-Chex CD4 Low and Normal samples were stained with CD3, CD4 and CD45, Lyse/wash
- Normal QC were acquired in triplicate followed by three replicates of CD4 low.

$$Carryover\ \% = [(B1\text{-}B3) / (A3\text{-}B3)] \times 100$$

A3 = Normal replicate 3; B1 = Low replicate 1, B3 = Low replicate 3.

Instrument Comparison with ERF Values

Parameter	Bead Population	Instrument 1 Calculated ERF	Instrument 2 Calculated ERF	Mean	St. Dev	%CV	% Bias
UV585	1	404,463	402,717	403,590	1,235	0.31	-0.43
	2	8,557,424	8,616,002	8,586,713	41,421	0.48	0.68
	3	562,393,116	560,991,553	561,692,335	991,054	0.18	-0.25
V510	1	52,557	52,604	52,580	33	0.06	0.09
	2	452,387	451,568	451,978	579	0.13	-0.18
	3	3,779,325	3,782,792	3,781,059	2,452	0.06	0.09
B576	1	248,239	248,696	248,467	323	0.13	0.18
	2	2,877,852	2,866,104	2,871,978	8,307	0.29	-0.41
	3	26,407,272	26,466,790	26,437,031	42,085	0.16	0.23
YG695	1	107,034	107,093	107,063	41	0.04	0.05
	2	1,418,393	1,416,578	1,417,485	1,284	0.09	-0.13
	3	13,147,956	13,157,624	13,152,790	6,836	0.05	0.07
R780	1	120,000	120,000	120,000	0	0.00	0.00
	2	1,471,628	1,471,628	1,471,628	0	0.00	0.00

- SRQP were acquired at optimal voltages on two instruments and ERF Values were calculated.
- One detector from each laser line is shown as an example.
- Note- Instrument cross-calibration is ongoing.

REFERENCES

1. CLSI. Validation of Assays Performed by Flow Cytometry. CLSI document H62. 1st Edition Wayne PA (Ed.). Clinical Laboratory Standards Institute (2021).
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