

The BD FACSLyric™ System and Assay Portability for Phenotyping T-, B- and Natural Killer Lymphocytes.

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Introduction

Immune phenotyping of the T-, B- and NK cells using flow cytometry has been a useful clinical laboratory methodology to assess status of the immune system in whole blood from patients known or suspected to have immunosuppressive clinical disease(s).

The BD FACSLyric™ system consists of a flow cytometer available in four different optical configurations, the BD FACSuite™ Clinical software, with optional BD FACST™ Universal Loader, and the BD FACSLink™ interface for transfer data to a Laboratory Information System. The BD FACSuite Clinical software when used with BD™ FC beads and BD™ CS&T IVD beads supports the universal set-up for performance QC, instrument control, data acquisition and storage, and online/offline data analysis, and instrument standardization.

The objective of this study was to demonstrate the degree of between laboratory precision of the BD FACSLyric 10-color configuration system using the BD Multitest™ 6-color TBNK with BD Trucount™ tubes supporting portability of the assay across multiple clinical laboratories.

Materials and Methods

Four laboratories participated in the study, these labs also participated in the Method Comparison of the FACSLyric clinical evaluation. The labs were located one in Germany (SYN) and three in USA (MED, UNC and UPM). Using the BD FACSLyric 10-color configuration to test the same lot of the two level of CD Chex control material, normal and low (Streck, La Vista, NE, USA). The samples were stained using the BD Multitest 6-color TBNK reagent with BD Trucount tubes (BD Biosciences, San Jose, CA, USA) to identify T-, B- and natural killer (NK) lymphocyte cells. Samples were analyzed in the BD FACSLyric 10-color flow cytometer with BD FACSuite Clinical software using FC and CS&T beads (BD Biosciences, San Jose, CA, USA). All the labs tested the same lot of the control material during five non-consecutive days of testing, with two runs per day and triplicate samples per run. The results were analyzed using percent coefficient of variation to determine total and between-site precision of the T-, B- and NK absolute counts (cells/μL) per site.

The results were analyzed per site to determine the percent of coefficient of variation (%CV) and standard deviation (SD) for the absolute count and the percentage of lymphocytes for T-, B- and NK cells.

Results

For the CD3+ T lymphocytes, %CV values for total precision were 3.5 to 6.8%, for within-run precision were between 3.4 to 6.4%. The CD3+CD4+ T cell %CV values were between 3.6 to 8.8% for total precision, and 3.4 to 7.2 for within-run precision. The %CV values for CD3+CD8 T lymphocyte were between 3.8 to 9.2 for total precision, and 3.5 and 7.3 for within-run precision. The details for the T cell types are shown for total precision (Table 1) and within-run precision (Table 2) for both CD Chex tested levels.

Table 1: T-, B- and NK Lymphocyte Absolute Count Total Precision.

Cells	Sample Site ID	Low				Normal			
		MED	SYN	UNC	UPM	MED	SYN	UNC	UPM
CD3	Variance	1407.0	1056.6	935.6	3410.9	7455.0	3705.2	8566.2	14217.6
	Mean	889.9	878.8	863.4	871.1	1762.7	1752.3	1697.0	1757.6
	SD*	37.5	32.5	30.6	58.4	86.3	60.9	92.6	119.2
	SD UL [#]	50.4	43.7	41.1	78.5	116.1	81.8	124.4	160.3
	%CV**	4.2	3.7	3.5	6.7	4.9	3.5	5.5	6.8
	%CV UL	5.3	4.7	4.5	8.5	6.2	4.4	6.9	8.6
CD4	Variance	188.4	160.9	110.5	256.4	3934.1	1795.5	4140.1	8322.4
	Mean	189.0	188.0	183.5	182.7	1192.9	1183.7	1143.6	1180.6
	SD*	13.7	12.7	10.5	16.0	62.7	42.4	64.3	91.2
	SD UL [#]	18.5	17.1	14.1	21.5	84.3	57.0	86.5	122.6
	%CV**	7.3	6.7	5.7	8.8	5.3	3.6	5.6	7.7
	%CV UL	9.2	8.5	7.2	11.1	6.7	4.5	7.1	9.8
CD8	Variance	960.4	572.7	651.1	1766.6	1266.7	554.9	1619.7	2514.8
	Mean	621.2	623.7	611.1	611.5	543.3	599.4	543.3	545.4
	SD*	31.0	23.9	25.5	42.0	35.6	23.6	40.2	50.1
	SD UL [#]	41.7	32.2	34.3	56.5	47.8	31.7	54.1	67.4
	%CV**	5.0	3.8	4.2	6.9	6.6	3.9	7.4	9.2
	%CV UL	6.3	4.9	5.3	8.7	8.3	5.0	9.4	11.6
CD19 B Cells	Variance	519.3	306.4	244.0	623.6	365.9	308.8	488.3	600.3
	Mean	339.7	339.4	324.2	336.9	281.5	276.7	277.0	270.9
	SD*	22.8	17.5	15.6	25.0	19.1	17.6	22.1	24.5
	SD UL [#]	30.6	23.5	21.0	33.6	25.7	23.6	29.7	32.9
	%CV**	6.7	5.2	4.8	7.4	6.8	6.4	8.0	9.0
	%CV UL	8.5	6.5	6.1	9.4	8.6	8.0	10.1	11.5
CD16+CD56 NK Cells	Variance	424.3	294.3	291.9	468.2	306.6	558.7	334.4	513.8
	Mean	296.3	303.7	288.9	289.1	239.6	233.7	229.4	221.5
	SD*	20.6	17.2	17.1	21.6	17.5	23.6	18.3	22.7
	SD UL [#]	27.7	23.1	23.0	29.1	23.5	31.8	24.6	30.5
	%CV**	7.0	5.6	5.9	7.5	7.3	10.1	8.0	10.2
	%CV UL	8.8	7.1	7.5	9.5	9.2	12.8	10.1	13.0

*SD= standard deviation; **%CV= percent coefficient of variation, *UL= Upper Limit.

Table 2: T-, B- and NK Lymphocyte Absolute Count Within-run Precision.

Cells	Source Site ID	Low				Normal			
		MED	SYN	UNC	UPM	MED	SYN	UNC	UPM
CD3	Variance	1198.6	908.8	935.6	3127.2	7455.0	3605.6	8216.5	10581.2
	Mean	889.9	878.8	863.4	871.1	1762.7	1752.3	1697.0	1757.6
	SD*	34.6	30.1	30.6	55.9	86.3	60.0	90.6	102.9
	SD UL [#]	50.0	43.5	44.2	80.8	124.7	86.7	130.9	148.5
	%CV**	3.9	3.4	3.5	6.4	4.9	3.4	5.3	5.9
	%CV UL	5.1	4.5	4.7	8.5	6.5	4.5	7.1	7.7
CD4	Variance	186.4	151.8	94.0	195.7	3934.1	1662.6	3777.9	6634.0
	Mean	189.0	188.0	183.5	182.7	1192.9	1183.7	1143.6	1180.6
	SD*	13.7	12.3	9.7	14.0	62.7	40.8	61.5	81.4
	SD UL [#]	19.7	17.8	14.0	20.2	90.6	58.9	88.8	117.6
	%CV**	7.2	6.6	5.3	7.7	5.3	3.4	5.4	6.9
	%CV UL	9.6	8.7	7.0	10.1	7.0	4.6	7.1	9.1
CD8	Variance	867.9	483.3	651.1	1565.8	1266.7	554.9	1590.2	1269.8
	Mean	621.2	623.7	611.1	611.5	543.3	599.4	543.3	545.4
	SD*	29.5	22.0	25.5	39.6	35.6	23.6	39.9	35.6
	SD UL [#]	42.5	31.7	36.8	57.1	51.4	34.0	57.6	51.5
	%CV**	4.7	3.5	4.2	6.5	6.6	3.9	7.3	6.5
	%CV UL	6.3	4.7	5.5	8.6	8.7	5.2	9.7	8.6
CD19 B Cells	Variance	519.3	306.4	244.0	444.4	360.0	247.8	488.3	471.5
	Mean	339.7	339.4	324.2	336.9	281.5	276.7	277.0	270.9
	SD*	22.8	17.5	15.6	21.1	19.0	15.7	22.1	21.7
	SD UL [#]	32.9	25.3	22.6	30.4	27.4	22.7	31.9	31.4
	%CV**	6.7	5.2	4.8	6.3	6.7	5.7	8.0	8.0
	%CV UL	8.9	6.8	6.4	8.3	8.9	7.5	10.6	10.6
CD16+CD56 NK Cells	Variance	424.3	294.3	291.9	403.3	306.6	546.9	303.8	481.9
	Mean	296.3	303.7	288.9	289.1	239.6	233.7	229.4	221.5
	SD*	20.6	17.2	17.1	20.1	17.5	23.4	17.4	22.0
	SD UL [#]	29.7	24.8	24.7	29.0	25.3	33.8	25.2	31.7
	%CV**	7.0	5.6	5.9	6.9	7.3	10.0	7.6	9.9
	%CV UL	9.2	7.5	7.8	9.2	9.7	13.3	10.1	13.1

*SD= standard deviation; **%CV= percent coefficient of variation, *UL= Upper Limit.

Figure 1: Between-laboratory Total and Within Run precision per Site.

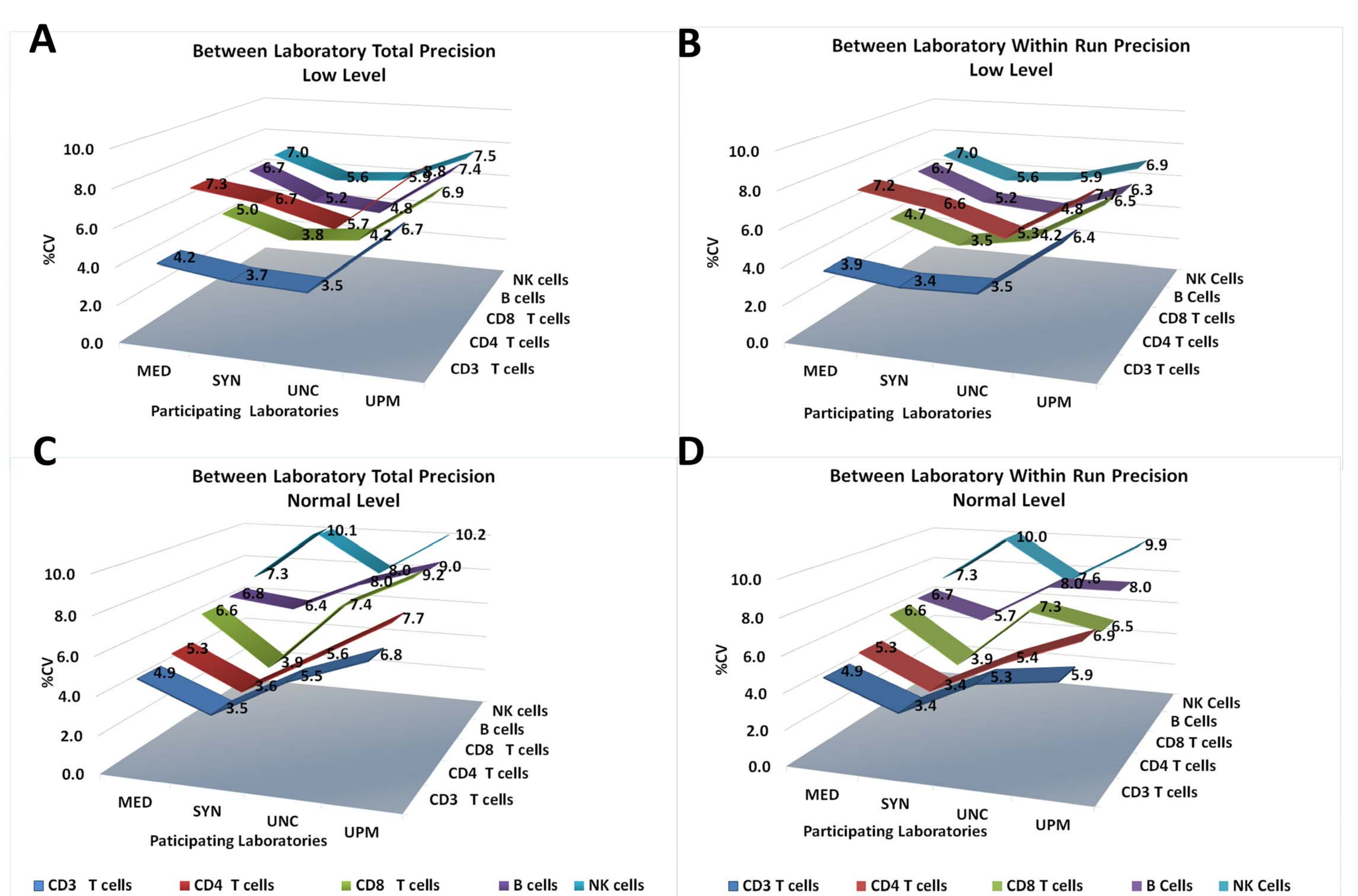


Figure 1. T-, B- and NK absolute count between laboratory Total and Within-Run Precision per site. A and B illustrate %CV results of the low level and C and D of the normal level of same lot of the control materials used as samples. A and C correspond to Total precision, and B and D to Within-run precision.

Discussion

The between site variability shows larger %CV in one lab, that may have been introduced by the operator skills. The rest of the results are closer for both, the low and normal samples. The BD FACSLyric system can provide accurate results for calculation of T-, B- and NK lymphocyte supporting portability of the assay across sites.

The BD FACSLyric System is for in vitro use only for 6-colors, and research use only for 7-10 colors. This product is CE Marked (IVD Directive 98/79/EC) in 2016.

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