Immunophenotyping of UK NEQAS CLL Samples on the BD FACSLyric™ and BD FACSCanto™ II

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Introduction

Flow cytometry is widely used in the field of hematologic neoplasms for the classification and quantitative characterization of aberrant phenotypes. Achieving consistent and reproducible cell analysis results is dependent on standardized procedures in flow cytometer setup/QC, in sample preparation using antibody reagents, and in data acquisition/analysis. The BD FACSLyric™ system simplifies standardization by enabling CS&T daily instrument setup and QC with automatic optimization of fluorescence compensation. Biological assays with the same tube or assay settings are transportable across different BD FACSLyric instruments for multisite studies. In this study we demonstrate methods to transfer assay settings from BD FACSCanto™ II to BD FACSLyric and compare results obtained on the two instruments using Chronic Lymphocytic Leukemia (CLL) samples provided by the United Kingdom National External Quality Assessment Service (UK NEQAS) consortium.

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Reagents

Panel A: One-tube reagent panel for lymphoid screening (CD8+Ig Lambda FITC, CD56+Ig Kappa PE, CD5 PerCp-Cy5.5, CD19+TCRgd PE-Cy7, CD3 APC, CD38 APC-H7, CD20+CD4 V450, CD45 V500)
The markers in Panel A were selected to correspond respectively to the EuroFlow™ Consortium's Lymphoid Screening Tube (LST)

Panel B: One-tube reagent panel for the detection of B-cell lymphoproliferative diseases (CD23 FITC, CD10 PE, CD79b PerCp-Cy5.5, CD19 PE-Cy7, CD200 APC, CD43 APC-H7, CD20+CD4 V450, CD45 V500)
The markers in Panel B were selected to correspond respectively to the EuroFlow™ Consortium's B-Cell Chronic Lymphoproliferative Diseases (B-CLPD) tube 1.

Instruments

BD FACSCanto II 8-color 4-2H-2V with FACSDiva™ software
BD FACSLyric 10-color 4-Blue 3-Red 3-Violet with FACSuite RUO software

Table 1 Procedures to transfer FACSCanto II setup to BD FACSLyric

	BD FACSCanto II with FACSDiva software	BD FACSLyric with FACSuite RUO software
Initial setup	1. If baseline is defined, run a performance check using BD FACSDiva CS&T IVD beads (Cat# 656046 or 656047) 2. Install Setup template and analysis template for the multi-color tube (Panel A and Panel B) 3. Adjust fluorescent PMT voltages using BD OneFLow Setup beads (Cat# 658620) with lot-specific MFI target ranges per the BD OneFlow™ target MFI Setup worksheet 4. Adjust the FSC and SSC voltages using normal lyse washed fresh whole blood within specified ranges per the BD OneFlow™ Scatter Setup worksheet 5. Create and save Application Settings 6. Use BD™ FC beads 8-color kit for BD OneFlow Assays (Cat# 658621) to generate SOV for fluorescence compensation 7. To prepare transferring FACSCanto setup to FACSLyric, use single FC beads as sample to measure MFI for 8 fluorescence channels: FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, APC-H7, V450, V500, as well as FSC, SSC (see Table 2)	a. If baseline is defined, run a performance check using BD CS&T (CE/IVD) beads (Cat# 656504 or 650505) b. Create setup and analysis templates in FACSuite for Panel A and Panel B c. Adjust fluorescent PMT voltages for each fluorescence channel using the same FC beads in step 7 to match with FACSCanto II MFI (run the same FC beads within 1 hour of preparation). See Table 2. d. Adjust PMTV for FSC, SSC using the same FC beads in step 7 to match with MFI on FACSCanto II. See Table 2. e. Create and save tube settings for Panel A and Panel B f. Create and save Assay Reference Settings using BD FC beads 7-color kit (CE/IVD) (Cat# 656867) and BD FC beads 5-color kit (Cat# 661564) g. Create and save two assays for Panel A and Panel B, respectively
Daily setup	• Run a performance check using BD FACSDiva CS&T IVD beads • Link to/unlink from compensation and apply Application Settings • Confirm that PMTVs remains within a range of +/-15% of the initial target MFI values	• Run a performance check using CS&T beads • Run Assay and Tube Settings Setup • Confirm that PMTVs remains within a range of +/-15% of the initial target MFI values

Table 2 MFI of FC beads after setup transfer

	MFI of FC beads *		
Channel	FACSCanto	FACSLyric	%Diff in MFI
FSC	57351	58413	1.9
SSC	12488	12321	-1.3
FITC	23850	23525	-1.4
PE	69421	69203	-0.3
PerCP-Cy5	36744	36564	-0.5
PE-Cy7	87522	87404	-0.1
APC	112971	113001	0.0
APC-H7	25998	25705	-1.1
V450	46831	46793	-0.1
V500	15612	15423	-1.2

*Note: FC beads were used as samples for each fluorescence channel. Data was from one representative setup run.

Fig. 1 Example dot plots of a CLL patient sample

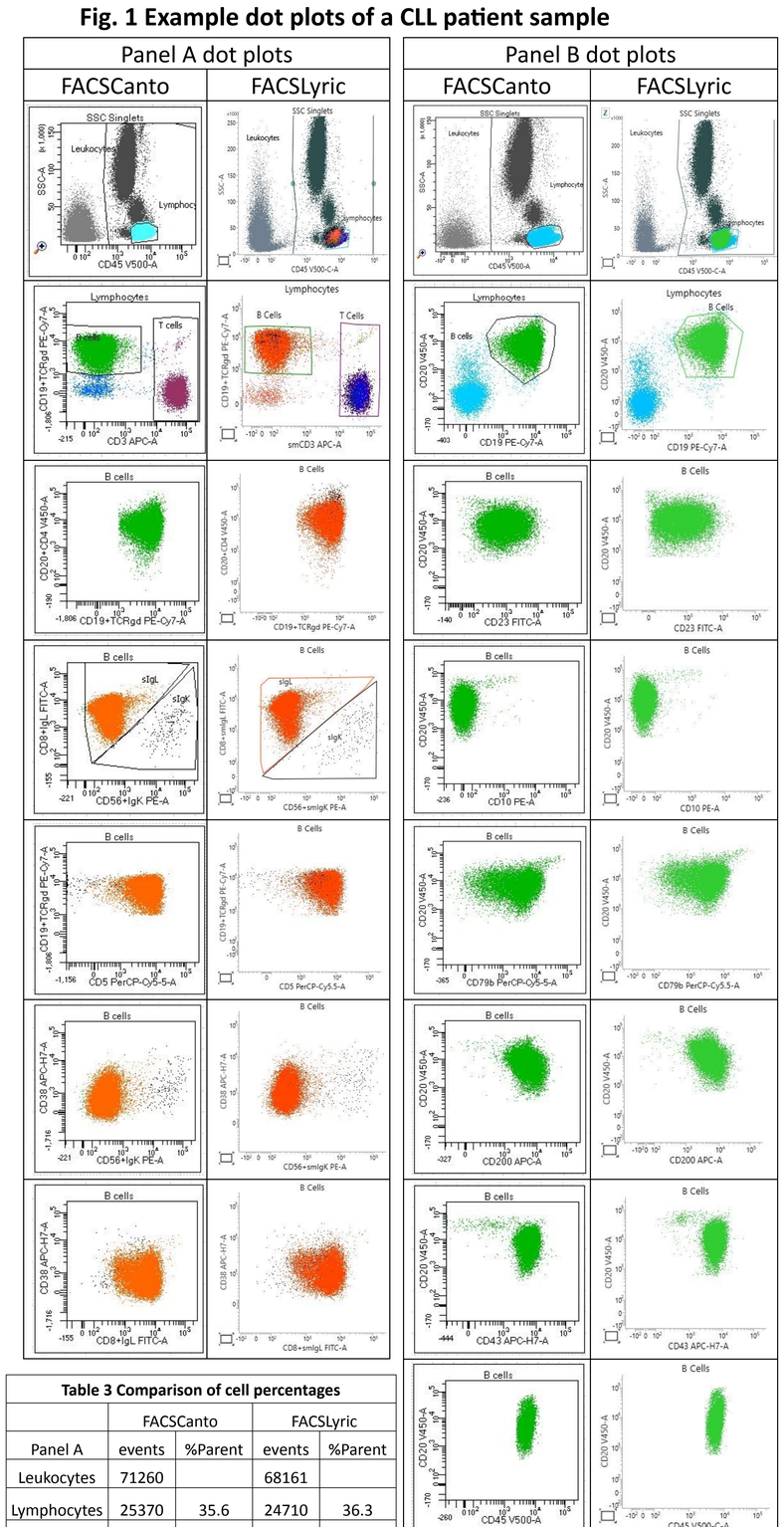

Table 3 Comparison of cell percentages

	FACSCanto		FACSLyric	
Panel A	events	%Parent	events	%Parent
Leukocytes	71260		68161	
Lymphocytes	25370	35.6	24710	36.3
T cells	6183	24.4	5938	24.0
TCRgd-	6064	98.1	5845	98.4
B cells	17943	70.7	17724	71.7
sIgL cells	17706	98.7	17512	98.8

Figure 1. Analysis of CLL patient sample stained with Panel A and Panel B
Instrument assay settings were established on FACSCanto II, then transferred to BD FACSLyric, as described in Table 1. One B-CLL patient sample was stained using Panel A and Panel B reagents and acquired on FACSCanto II and FACSLyric. Analysis dot plots demonstrated typical Immunophenotyping profiles for B-CLL that were comparable between the two instruments after setup transfer. Example cell percentages (Panel A) obtained on the two instruments are shown in Table 3.

Fig. 2 Example dot plots of UK NEQAS B-CLL Sample

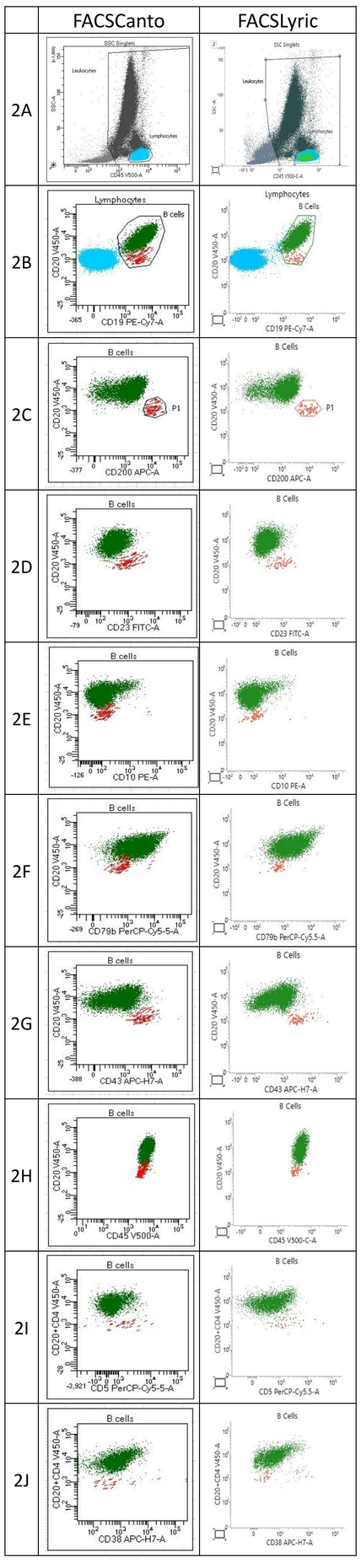

Figure 2. UK NEQAS CLL sample stained with Panel A and Panel B to identify abnormal B cells

Note: Medium-sized red dots show abnormal B cells in the dot plots on BD FACSCanto II. Highlighted red dots show abnormal B cells in dot plots on FACSLyric. .

2A to 2H: Panel B, 500,000 total events were collected. Abnormal B cell event number was 89 on FACSCanto, 68 on FACSLyric.

2I and 2J: Panel A, 200,000 total events were collected. Abnormal B cell event number was 26 on FACSCanto , 24 on FACSLyric.

	MFI of abnormal B cells (P1 gate)		
Panel B	FACSCanto	FACSLyric	%Diff
CD23 FITC	1079	903	-16.3
CD10 PE	125	125	0.0
CD79b PerCP-Cy5-5	671	631	-6.0
CD19 PE-Cy7	3311	3191	-3.6
CD200 APC	10935	11407	4.3
CD43 APC-H7	4434	3317	-25.2
CD20 V450	1175	1165	-0.9
CD45 V500	3393	3182	-6.2

Conclusion

Chronic Lymphocytic Leukemia (CLL) samples can be analyzed on the FACSLyric for standardized and consistent results as compared with FACSCanto II and peer laboratories.

For research use only.

Not for use in diagnostic or therapeutic procedures.

Class 1 Laser Product.

UK NEQAS samples and preparation

We received UK NEQAS CLL samples #19 and #20 from UK NEQAS BCLL program and analyzed them using Panel A and Panel B reagents on BD FACSCanto II and BD FACSLyric instruments in our lab, as shown in Figure 2. Instruments were set up using procedures described in Tables 1. The samples was manufactured by UK NEQAS using CLL patient samples and stabilized whole blood units.

Preparation and staining: For each specimen, wash 300 uL of specimen with 10 mL of wash buffer (filtered PBS + 0.5% BSA + 0.1% sodium azide) by inverting the tube 3–5 times to mix well. Centrifuge at 540g for 5 minutes at 20°C–25°C (RT). Remove the supernatant and resuspend the cell pellet. Repeat wash process three times. Resuspend the cell pellet in 200 µL of wash buffer. Add 100 µL of washed patient specimen to a tube with Panel A (or Panel B) reagents and mix well. Incubate for 30 minutes at RT, protected from light. Add 2 mL of 1X BD FACS lysing solution to each tube and mix well. Incubate for 10 minutes at RT, protected from light. Centrifuge at 540g for 5 minutes at RT. Remove the supernatant and resuspend the cell pellet. Add 2 mL of wash buffer (filtered PBS + 0.5% BSA + 0.1% sodium azide) to each tube and mix well. Centrifuge at 540g for 5 minutes at RT. Remove the supernatant and resuspend the cell pellet. Add 200 µL of wash buffer to each tube and mix well. Acquire the stained samples within 1 hour of staining. Store the stained sample at 2°C–8°C in the dark until acquisition.

Acquisition and analysis

The stained and prepared UK NEQAS CLL samples #19 and #20 were acquired on BD FACSCanto II and BD FACSLyric at medium flow rate with threshold on FCS 10,000. The acquisition target was set to acquire 200, 000 total events. The percentage of abnormal B cells relative to total white blood cells was calculated, as shown in Table 3. We compared percentages of abnormal B cells on BD FACSCanto II and BD FACSLyric relative to UK NEQAS reports using z score analysis. The number of laboratories that used BD FACSCanto II cytometers in the UK NEQAS trial are shown in Tables 3. Robust mean and standard deviation of abnormal B cell percentages were provided by the UK NEQAS trial reports reflecting statistical results of peer laboratories.

z score of abnormal B cells% for the BD FACSLyric (or FACSCanto) is defined as
z = (measured %abnormal B cells — robust mean of %abnormal B cells of peer laboratories)/Standard deviation of % abnormal B cells of peer laboratories

Fig. 3 Histograms of participant z scores on percentage of abnormal B cells

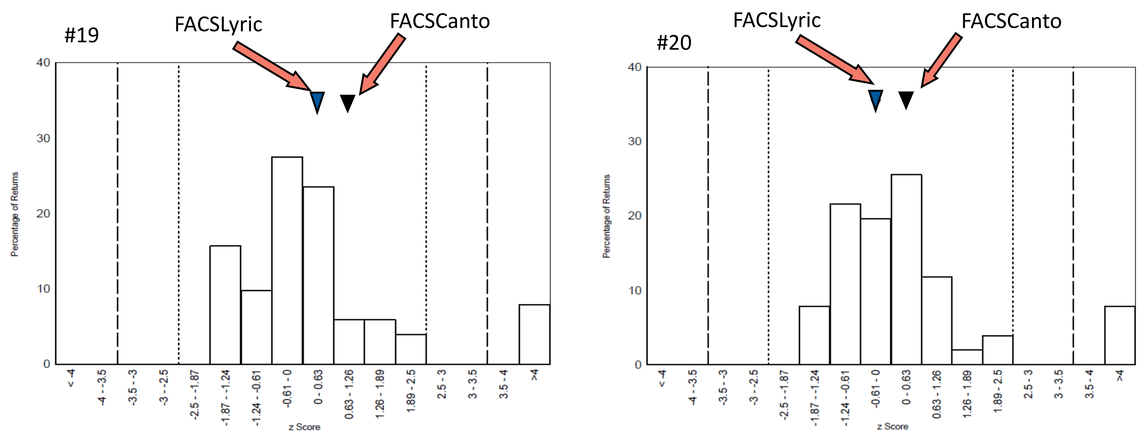

Table 4 Comparison of percent abnormal B cells between FACSLyric and UK NEQAS results

	UK NEQAS trial statistics			BD results			
	%abnormal B cells on FACSCanto II			FACSCanto II		FACSLyric	
Sample	No. Labs	Robust Mean	SD	% Abnormal B cells	z-score	% Abnormal B cells	z-score
#19	33	0.0390	0.0237	0.0593	0.857	0.0419	0.122
#20	32	0.0518	0.0244	0.0523	0.020	0.0426	-0.377

Discussion

In this study, we demonstrated procedures (Table 1 and Table 2) to establish initial setup on FACSCanto II and transfer to BD FACSLyric. This setup transfer method generated comparable Immunophenotyping profiles on FACSCanto II and FACSLyric for cells stained with reagents Panel A and Panel B, as shown in Figure 1 using a CLL patient sample. We further tested two UK NEQAS CLL samples on FACSCanto II and FACSLyric after setup transfer to identify abnormal B cell populations in the sample, as shown in Figure 2. We calculated percentages of abnormal B cells relative to total white blood cells for the two UK NEQAS CLL samples and compared with results of peer laboratories, as shown in Table 4 and Figure 3. Z score statistics demonstrated that UK NEQAS CLL samples analyzed using Panel A and Panel B on FACSCanto II and FACSLyric gave comparable results. Relative to peer laboratories, both FACSCanto II and FACSLyric generated satisfactory results in the UK NEQAS BCLL program.