# Performance Evaluation in Europe of the BD FACSLyric™ 10-Color System Using Remnant Specimens with BD Tritest™ Reagents

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## Introduction

The BD FACSLyric™ system consists of a flow cytometer available in different optical configurations, BD FACSuite™ Clinical software, the optional BD FACS™ Universal Loader, and the optional BD FACSLink™ interface for data transfer to a Lab Information System (LIS). BD FACSuite Clinical software, used with BD™ FC beads and BD™ CS&T beads, supports IVD universal setup (performance QC, instrument control), data acquisition and storage, and on/off-line data analysis.

BD carried out a performance evaluation with BD Tritest™ reagents: CD3/CD4/CD45 and CD4/CD8/CD3. The objective was to determine the expected difference between the BD FACSLyric 10-color system and predicate IVD systems for measuring absolute lymphocyte subset counts and percentages of the lymphocyte subpopulations.

- BD Tritest™ CD3/CD4/CD45 reagent: CD3, CD4, %CD3, %CD4
- BD Tritest™ CD4/CD8/CD3 reagent: CD3, CD4, CD8, %CD4, %CD8

## Materials and Methods

A performance evaluation was conducted with the BD FACSLyric 10-color configuration using de-identified and delinked remnant venous blood specimens from HIV-infected and uninfected patients attending for routine laboratory testing. The samples were prepared using BD Tritest CD3/CD4/CD45 and BD Tritest CD4/CD8/CD3 reagents with BD Trucount™ tubes and BD Trucount™ controls. Samples were tested using the BD FACSCalibur™ system with BD Multiset™ software, and the BD FACSLyric system with BD FACSuite Clinical software, using FC and CS&T beads. The data was analyzed for mean percent biases of the absolute counts/µL and percentages of lymphocytes for the different lymphocyte subsets for each BD Tritest reagent using Deming regression. Bland-Altman plots were obtained, and agreement analysis at the cutoffs of 350 and 200 cells/µL was carried out.

## Results

The total numbers of specimens enrolled per reagent were 106 for BD Tritest CD3/CD4/CD45 and 121 for BD Tritest CD4/CD8/CD3. Deming regression results gave $R^2$ ≥0.94, and slope estimated values were between 0.985 and 1.045 (Table 1). Results for lymphocyte subset absolute counts and percentages per reagent are summarized in Table 1.

**Table 1: Summary of Deming Regression results per BD Tritest reagent**

| BD Tritest reagent | Lymphocyte subset | | $R^2$ | Slope [95% CI*] | Intercept |
|---|---|---|---|---|---|
| CD3/CD4/CD45 | Absolute counts (cells/µL) | CD3+ | 0.97 | 0.985 [0.956 to 1.014] | 63.32 |
| | | CD4+ | 0.98 | 1.023 [1 to 1.045] | 2.25 |
| | Percentage of lymphs (%) | %CD3 | 0.94 | 1.011 [0.978 to 1.045] | 0.13 |
| | | %CD4 | 0.98 | 1.02 [0.993 to 1.048] | -0.26 |
| CD4/CD8/CD3 | Absolute counts (cells/µL) | CD3+ | 0.96 | 1.022 [0.986 to 1.059] | 44.44 |
| | | CD4+ | 0.98 | 1.043 [1.016 to 1.071] | 3.83 |
| | | CD8+ | 0.96 | 1.045 [1.011 to 1.079] | 2.01 |
| | Percentage of lymphs (%) | %CD4 | 0.99 | 0.993 [0.973 to 1.014] | 0.14 |
| | | %CD8 | 0.96 | 1.002 [0.976 to 1.028] | -0.44 |

*CI= Confidence Interval*

Table 2 summarizes the mean percent bias with the lower and upper confidence limits for each lymphocyte subset per reagent.

**Table 2: Percentage % mean bias per BD Tritest reagent**

| Reagent | Parameter | N | AbsCD3 | %CD3 | AbsCD4 | %CD4 | AbsCD8 | %CD8 |
|---|---|---|---|---|---|---|---|---|
| TT CD3/ CD4/CD45 | %Bias (LCL, | 106 | 3.06 (1.96, 4.16) | 1.33 (0.67, 2.00) | 2.9 (1.6, 4.21) | 1.20 (0.14, 2.26) | NA | NA |
| TT CD4/ CD8/CD3 | %Bias (LCL, | 121 | 5.45 (3.86, 7.05) | NA | 5.70 (4.33, 7.07) | -0.24 (-0.94, 0.46) | 5.10 (3.60, 6.60) | -0.54 (-1.23, 0.15) |

**LCL or UCL= Lower Confidence Limit or Upper Confidence Limit*

Bland-Altman and Deming regression graphs per BD Tritest reagent are illustrated in Figure 1. Table 3 shows results from the method agreement around the CD4 clinically relevant cutoffs (200 and 350 cells/µL), and Table 4 summarizes results from predicted bias intervals at the CD4 clinical cutoff (200 cells/µL).

*Note: due to the limited number of specimens enrolled around the clinical cutoffs, the confidence limit range is wide.*

**Figure 1: Bland-Altman and Deming regression per BD Tritest reagent**

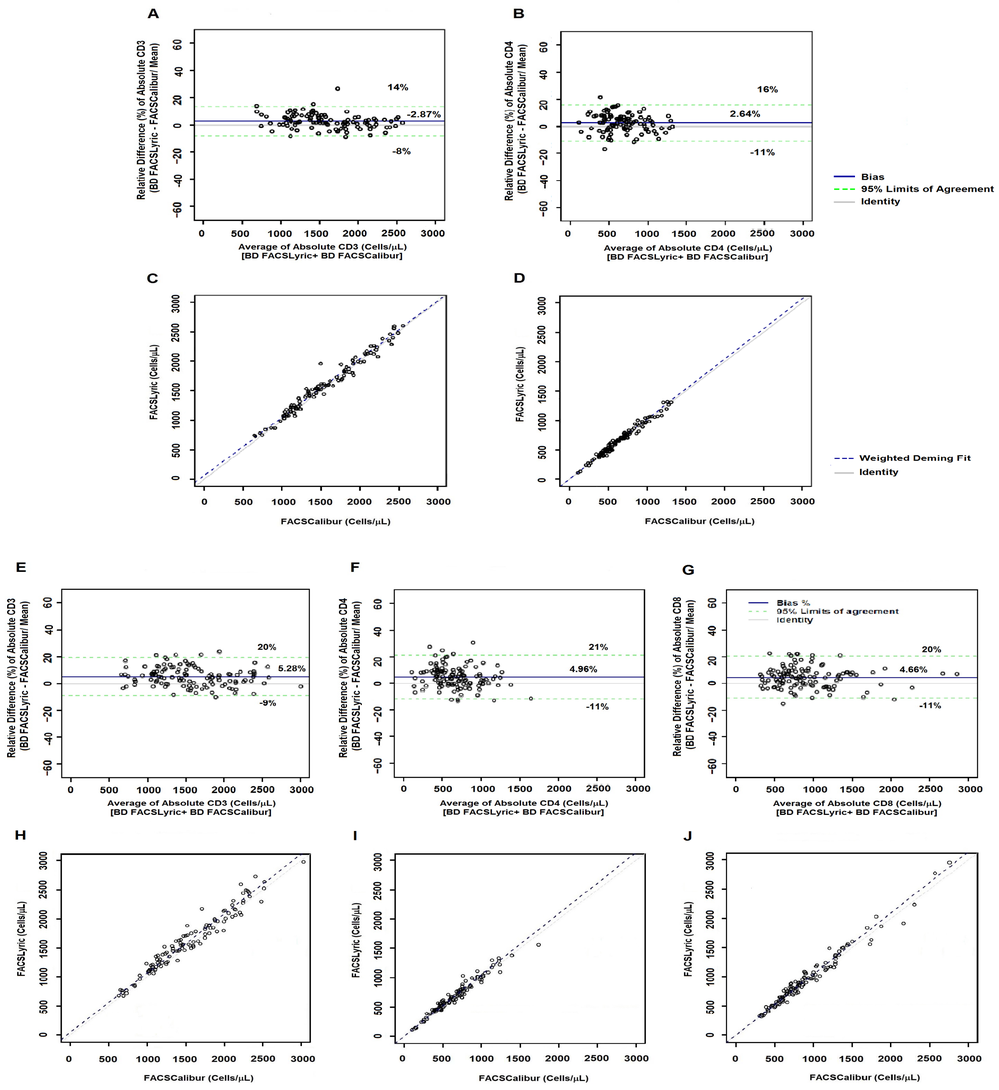


**Figure 1.** BD Tritest CD3/CD4/CD45 Bland-Altman plots (A, B) and Deming regression graphs (C, D) and BD Tritest CD4/CD8/CD3 Bland-Altman plots (E, F, G) and Deming regression graphs (H, I, J).

**Table 3: Agreement around the CD4 clinical cutoffs of 200 and 350 cells/µL**

| AbsCD4 cutoff | BD Tritest reagent | Agreement | FACSLyric (N) | FACSCalibur (N) | % Agreement | LCL** | UCL** |
|---|---|---|---|---|---|---|---|
| 200 cells/ µL | CD3/CD4/CD45 | Overall | 106 | 106 | 100% | 96.50% | 100% |
| | | Positive | 2 | 2 | 100% | 34.24% | 100% |
| | | Negative | 104 | 104 | 100% | 96.44% | 100% |
| | CD4/CD8/CD3 | Overall | 121 | 121 | 100% | 96.92% | 100% |
| | | Positive | 4 | 4 | 100% | 51.01% | 100% |
| | | Negative | 117 | 117 | 100% | 96.82% | 100% |
| 350 cells/ µL | CD3/CD4/CD45 | Overall | 105 | 106 | 99.06% | 94.85% | 99.83% |
| | | Positive | 8 | 9 | 88.89% | 56.50% | 98.0% |
| | | Negative | 97 | 97 | 100% | 96.19% | 100.0% |
| | CD4/CD8/CD3 | Overall | 118 | 121 | 97.52% | 92.96% | 99.15% |
| | | Positive | 10 | 13 | 76.92% | 49.74% | 91.82% |
| | | Negative | 108 | 108 | 100% | 96.57% | 100% |

**LCL or UCL= Lower Confidence Limit or Upper Confidence Limit*

**Table 4: Predicted bias interval at CD4 clinical cutoffs by BD Tritest reagent**

| BD Tritest reagent | CD4 cutoff (cells/µL) | Bias (cells/µL) | 95% CI | %Bias (%) | 95% CI |
|---|---|---|---|---|---|
| CD3/CD4/CD45 | 200 | 6.78 | -1.20, 14.75 | 3.39 | -0.60, 7.38 |
| | 350 | 10.18 | 3.59, 16.78 | 2.91 | 1.03, 4.79 |
| CD4/CD8/CD3 | 200 | 12.53 | 4.65, 20.41 | 6.26 | 2.32, 10.21 |
| | 350 | 19.05 | 12.38, 25.72 | 5.44 | 3.54, 7.35 |

## Discussion

This performance evaluation shows that the BD FACSLyric system performance is equivalent to the performance of the BD FACSCalibur system with BD Tritest reagents with BD Multiset software. The BD FACSLyric system provides accurate results for calculation of lymphocyte subsets in remnant venous blood.

The BD FACSLyric System is not available for sale in USA.

This product is CE Marked (IVD Directive 98/79/EC).