Daily Quality Control in CD3\(^+\) and CD4\(^+\) T Cell Estimation by the FACSCount System at a Tertiary Care Center in South India

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CD4\(^+\) T cell count estimations are subject to high variations; hence, in this study, the previous day's tested samples were included routinely as the internal quality controls. The percentages of variation of the 2-day values were analyzed for 280 observations and the mean variation for CD4\(^+\) and CD3\(^+\) T cell counts ranged from 5.21% to 9.66%. This method is a good internal quality control (IQC) procedure for the estimation of CD3\(^+\) and CD4\(^+\) T cell counts in resource-poor settings.

The absolute CD4\(^+\) T-cell count is an important laboratory tool for monitoring HIV-infected individuals (5). Frequent monitoring of CD4\(^+\) T lymphocytes is essential to assess the immune suppression and the disease progression of HIV-infected individuals (10, 3). This also helps physicians to decide when to initiate antiretroviral therapy (ART) (4, 2) and when to change therapy due to immunologic failure in resource-poor settings (7, 11). There are numerous factors that are associated with T-cell count, with the standard technique used being flow cytometry (1). There are numerous factors that are associated with variation in the absolute lymphocyte subset counts estimated (6). The reported mean variation of CD4\(^+\) T-cell count, with the standard technique used being flow cytometry (1). There are numerous factors that are associated with variation in the absolute lymphocyte subset counts estimated (6). The reported mean variation of CD4\(^+\) T-cell counts in a "single-platform" methodology is about 13.7% (range, 10% to 18.3%). The variation reported for "double-platform" systems ranges from 14.5% to 43.4% (mean, 23.4%) (1).

Since the CD4\(^+\) T cell estimation is subject to a large amount of variation, it is very important that daily quality control measures are carried out in the laboratory. In its guidelines, the National AIDS Control Organization (NACO), India, has suggested for all the laboratories to do daily quality control testing that incorporates two samples (the samples with the lowest and highest CD4\(^+\) cell counts) that had been tested the previous day.

In this study, we investigated the performance of CD4\(^+\) T cell count estimation using the FACSCount system (Becton, Dickinson) by retrospectively analyzing the results of the two daily quality control samples.

The CD3\(^+\) and CD4\(^+\) T cell estimations were carried out using the FACSCount system (Becton, Dickinson) as reported earlier (10). In our laboratory, the testing was carried out from Monday through Friday and two internal quality control (IQC) samples were run every day except Mondays. The previous day's samples with the lowest and the highest CD4\(^+\) T cell counts were included as the controls. The samples were stored at room temperature (between 20 and 28\(^\circ\)C). The IQC values of a given day were compared with the previous day's values, and percent variation values were calculated. Percent variation was calculated as follows: (count on the first day/count on the second day) \(\times 100\). The data from July 2010 through January 2012 were analyzed. In the last month of the study, the laboratory also started using the Multi-Check control (Becton, Dickinson, San Jose, CA) for routine quality control testing. The data were also analyzed. A sample showing more than 20% variation from the previous day's value was considered not acceptable, and the clinical samples were retested if necessary.

Statistical analysis. The correlations of the counts on both the days were analyzed by Pearson's correlation test and regression analysis. Statistical analysis was done using Medcalc software version 9.2.0.1. The Bland and Altman plot analysis was also carried out between the first- and second-day values for both low CD3\(^+\)/CD4\(^+\) and high CD3\(^+\)/CD4\(^+\) counts.

A total of 280 observations were analyzed in the 19-month study period. The mean, median, standard deviation, correlation coefficient \(r^2\), and \(P\) values of both CD3\(^+\) and CD4\(^+\) T cell counts for the low and high IQCs are given in Table 1. The mean variations for the low CD3\(^+\) and low CD4\(^+\) T cell counts were 6.03% and 9.66%, respectively. The mean variations for the high CD3\(^+\) and high CD4\(^+\) T cell counts were 5.21% and 5.73%, respectively. The mean variations for the high CD3\(^+\) and CD4\(^+\) T cell counts above 1,000 cells/\(\mu\)l were 6.52% and 6.56%, respectively. The correlations of the low CD3\(^+\)/CD4\(^+\) and high CD3\(^+\)/CD4\(^+\) T cell counts estimated for the 2-day comparisons were significant (\(P < 0.001\)), with correlation coefficient \(r^2\) values ranging from 0.94 to 0.99.

The correlation coefficients of both low and high CD3\(^+\) and CD4\(^+\) IQCs were plotted as a graph as shown in the Fig. 1, and the results were significant. The Bland and Altman plot calculated for comparisons between the first- and second-day values obtained for low CD3\(^+\)/CD4\(^+\) and high CD3\(^+\)/CD4\(^+\) is shown in Fig. 2. The bias obtained for the low IQC for CD4 was 1.9 cells (95% confidence interval [CI], 30 to \(-26\)) and for CD3 was 2.2 cells (95% CI, 21 to \(-16\)). The bias obtained for the high IQC for CD4 was 2 cells (95% CI, 17 to \(-13\)) and for CD3 was 1.5 cells (95% CI, 17 to \(-14\)). The observed mean variation values for both CD4\(^+\) (12.4%) and CD3\(^+\) (23.95%) T cells were high when the cell count was below the linearity of the reportable ranges of 50 cells/\(\mu\)l for CD4\(^+\) T cells and 100 cells/\(\mu\)l for the CD3\(^+\) T cells. For all the
other ranges of cell counts, the variation was always below 10%. The data on the mean percent variation values obtained for different ranges of both CD4⁺ and CD3⁺ T cells are shown in Fig. 3.

Both high and low CD3⁺/CD4⁺ T cell count IQCs showed >20% variation on several occasions. The low CD4⁺ T cell counts showed >20% variation on 20 (3.6%) occasions (13 times when CD4 count was below 50 cells/µl, lower than the linearity of the assay), while the high CD4⁺ T cell counts showed >20% variation on 6 occasions (2.2%). The variation of low CD4⁺ T cell counts determined by excluding the 13 observations which were below the linearity of the assay was 7.9%. The low CD3⁺ T cell counts showed >20% variation on 3 different occasions (1.1%).

The data reported here give information about the daily quality control performance (precision) of CD3⁺/CD4⁺ T cell count estimations. Absolute CD4⁺ T cell count estimates can differ greatly between laboratories and within laboratories, as there are several factors which influence the CD4⁺ T cells (6). Hence, it is important to have a robust quality control system in place on a daily basis for all CD4 testing laboratories, apart from participating in a proficiency testing program. The reported average interlaboratory coefficient of variation (CV) for “single-platform” systems is 13%, and the nominal CD4⁺ T cell value of 300 cells/µl can range from 260 to 340 cells/µl. In our study, we looked only at the intralaboratory variation and the mean variations observed for the low CD3⁺ and low CD4⁺ T cell counts were 6.03% and 9.66%, respectively. The mean variations for the high CD3⁺ and high CD4⁺ T cell counts were 5.21% and 5.73%, respectively. The percent variations observed in our intralaboratory analysis were far lower than the reported mean interlaboratory variation of 13%. Earlier, when we compared the CD4⁺ T cell counts estimated by the FACSCount and GUAVA Easy CD4 systems, the mean variation, the bias, and the correlation observed between the two systems

### TABLE 1

| Parameter | Low CD3 (n = 276) | High CD3 (n = 275) | Low CD4 (n = 280) | High CD4 (n = 275) |
|-----------|-------------------|-------------------|-------------------|-------------------|
|           | 1st day | 2nd day | 1st day | 2nd day | 1st day | 2nd day | 1st day | 2nd day |
| Mean      | 767     | 759     | 2,309   | 2,298   | 90      | 88      | 856     | 841     |
| SD        | 466     | 453     | 682     | 672     | 79      | 79      | 307     | 308     |
| Median    | 691     | 710     | 2,205   | 2,220   | 74      | 71      | 805     | 779     |

- Correlation coefficient (r²) values for low CD3, high CD3, low CD4, and high CD4, 0.99, 0.94, 0.98, and 0.95, respectively; r² P values for low CD3, high CD3, low CD4, and high CD4, <0.001, <0.001, <0.001, and <0.001, respectively.
- Tube failure occurred four times; hence, data could not included for analysis.
- Tube failure occurred five times; hence, data could not included for analysis.

![FIG 1](https://example.com/figure1.png)

**FIG 1** Regression analysis of the CD3⁺ T and CD4⁺ T cell counts obtained with correlation coefficient values (r²). (a) IQC low CD3⁺ T cells; (b) IQC high CD3⁺ T cells; (c) IQC low CD4⁺ T cells; (d) IQC high CD4⁺ T cells.
were 12.9%, 64 cells/μl, and \( r \) of 0.96, respectively (9). In that reported study, there was a very good correlation between the cell counts observed on the first and the second days, with \( r^2 \) ranging from 0.94 to 0.99 (\( P < 0.001 \)). The Bland and Altman analysis also showed a very good correlation of the cell counts from the two days, with a bias ranging from 1.5 to 2.2 cells.

When individual day-to-day values were taken, there were a total of 20 occasions when the variation of the low CD4\(^+\) T cell count was more than 20%. On 13 of the 20 occasions, the CD4\(^+\) values were lower than the linearity range (50 cells/μl to 2,000 cells/μl). This would have also reflected in the higher mean percent variation values for low CD4 cells. On 2 of the 11 occasions when the low CD3\(^+\) T cell counts showed >20% variation, the cell counts were lower than the linearity range (100 cells/μl to 3,500 cells/μl). When we analyzed the percent variation and the cell count range, the maximum (>10%) variation was observed when the CD3\(^+\) T cell count was less than 50 cells/μl and when the CD4\(^+\) T cell count was <100 cells/μl. In this study, when we calculated the mean variation of CD4\(^+\) T cell counts (9.56%), we did not exclude any samples which were not in the linearity of the assay. When we recalculated the percent variation of low CD4\(^+\) T cells by excluding the 13 observations which were below the linearity of the assay, the mean variation was 7.9%. Hence, all the previously tested samples that are taken as IQC should have their cell counts within the respective linearity range. On 6 occasions, the high CD4\(^+\) T counts showed >20% variation. When this >20% variation happened, clinical samples were retested when they did not correlate with the previous cell counts or clinical condition of the individuals. There were a total of 35 clinical samples retested during the 19-month period, and of those, 31 (89%) showed a CV of <20% (27 samples showed <10% variation) with respect to the first day’s result. The other 4 (11%) samples from patients showed >20% variation. In these situations, we have looked at the earlier CD4\(^+\) T cell counts (approximately 6 months prior to the current testing) and at the clinical status of the patients, including treatment history. By analyzing these factors, the CD4 counts were correlated with the second day’s CD4\(^+\) T cell count.

In the last month of the study period, we also started using the Multi-Check Control (whole-blood control for lymphocyte subset enumeration; Becton, Dickinson, San Jose, CA) as a part of the quality control program. The expected count ranges for CD3\(^+\) and CD4\(^+\) T cells for the lot used were 792.6 to 1,188.9 and 503.8 to 755.7, respectively. In all the 25 observations for the Multi-Check control in that month, the cell counts for both CD3\(^+\) and CD4\(^+\) were within the expected range. The observed mean variations for the CD3\(^+\) and CD4\(^+\) T cells were 3.64% (range, 0.6% to 10.47%) and 4.96% (range, 0.03% to 8.87%), respectively. Those values were calculated in comparison to the expected absolute counts of 991 cells/μl and 630 cells/μl, respectively, for CD3\(^+\) and CD4\(^+\) T cells provided by the manufacturer. However, based on these 25 observations used when we calculated the precision, the value was about 3% (range, 0.05% to 6.5%) for both CD4\(^+\) and CD3\(^+\) T cells.

**FIG 2** Bland and Altman plot showing the first- and second-day CD3\(^+\) and CD4\(^+\) T cell counts for the low and high IQC samples. (a) IQC low CD3\(^+\) T cells; (b) IQC high CD3\(^+\) T cells; (c) IQC low CD4\(^+\) T cells; (d) IQC high CD4\(^+\) T cells. SD, standard deviation.
accuracy or bias. Since the ideal internal quality control to be used is a stabilized whole-blood sample, flow cytometry reagent manufacturers should consider supplying stabilized internal control samples along with the reagents, especially in resource-poor settings, for better quality control of the testing. On a particular day when there is a variation of >20% for these IQCs, counts obtained for routine clinical samples on that particular day should be scrutinized carefully before reporting and probably repeat tested. This practice would also increase the confidence of the technologist who is carrying out the test. However, it would be very helpful if there were a standard guideline on usage of previously tested samples as IQCs, the expected variation, and, if measured values deviate beyond the variation, how to validate the assay.

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