NOTE

CD4 Stabilization Tubes Provide Improved Accuracy of Absolute CD4 T-Cell Counts Compared to Standard K3 EDTA Tubes in Human Immunodeficiency Virus Immunologic Monitoring in Resource-Poor Settings

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CD4 stabilization tubes have the ability to ensure internal quality control in the human immunodeficiency virus (HIV) monitoring laboratory by maintaining accurate absolute CD4 T-cell counts for up to 6 days. Here, we assessed this technology for its use in an HIV clinical monitoring laboratory in a resource-poor setting in rural Uganda.

CD4 stabilization tubes (ST [Vacutainer CD4 stabilization blood collection tubes; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ]) have been developed as a means of extending preanalytic capacity and internal quality control for CD4 cell counts, improving reproducibility, and maintaining the accuracy of CD4 results for HIV-positive patients, thereby decreasing the overall laboratory costs associated with monitoring patients by reducing the number of rejected samples which require retesting. According to the manufacturer, ST are designed to preserve peripheral blood samples’ quantitative and qualitative leukocyte subset characteristics over time, thus dramatically reducing the concern for climactic factors and postcollection storage and/or transport time. The need for rebleeding patients due to sample loss could be dramatically reduced, as samples can be stored in ST at 37°C for up to 3 days or 30°C for up to 7 days according to the manufacturer’s standard operating procedure.

In order to assess these performance characteristics of ST in our setting, 59 (40 female and 19 male) HIV-positive individuals were recruited. After giving informed consent, volunteers at rural field clinics were bled aseptically into one standard K3 EDTA tube (BD Vacutainer; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and one ST per subject. Whole-blood samples were inverted 10 times for mixing and then prepared for analysis by the addition of fixative solution and fluorochrome-labeled antibody reagent pairs (anti-CD3 and anti-CD4 antibodies). Analysis was conducted on a FACSCount flow cytometer with software (version 1.4; both from Becton Dickinson, San Jose, CA) for the determination of absolute CD4 T-cell counts according to the manufacturer’s standard operating procedure. Daily FACSCount controls were analyzed prior to the runs, and Levy-Jennings charts were maintained for assessing linearity.

A total of 472 observations from repeated CD4 cell counts over time were obtained, and a total of 431 (91%) of 472 CD4 T-cell count results were obtained on day 0. ST were stored in vertical tube racks at ambient temperatures (25 to 28°C, as monitored by daily temperature recordings) and subsequently analyzed daily for 8 days.

Each day, whole-blood samples were inverted >10 times for mixing and then prepared for analysis by the addition of fixative solution and fluorochrome-labeled antibody reagent pairs (anti-CD3 and anti-CD4 antibodies). Analysis was conducted on a FACSCount flow cytometer with software (version 1.4; both from Becton Dickinson, San Jose, CA) for the determination of absolute CD4 T-cell counts according to the manufacturer’s standard operating procedure. Daily FACSCount controls were analyzed prior to the runs, and Levy-Jennings charts were maintained for assessing linearity.

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Novel technologies for improving internal quality control are needed in human immunodeficiency virus (HIV) monitoring laboratories in resource-poor settings. Accurate CD4 T-cell counts are a valuable tool to ensure accurate immunologic monitoring of HIV-positive patients receiving antiretroviral treatment (ART) (1). In Uganda, several factors can influence the quality of blood samples submitted for testing. In our setting, temperatures can reach up to 28°C and samples can require storage and/or transport for several hours before laboratory processing and analysis.

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cell counts over the 8 days were used for this analysis. Nine of the 59 patients were already on ART at the time of bleeding. For patients in our cohort, ART was initiated at a CD4 cell count of 250 cells/µl, hence the small number of specimens in our sample with very low CD4 cell readings (range, 58 to 948 cells/µl).

We used generalized linear models to obtain regression coefficients and mean CD4 cell counts as intercepts. Since the same sample was subjected to repeated counts of CD4 cells over time, we adjusted for the correlated data in this model to estimate robust standard errors of the regression coefficients by using the cluster and robust commands in STATA (version 9.2; Stata Corporation, College Station, TX), and 95% confidence intervals (CI) for the estimates were obtained.

The rate of decline in the CD4 cell counts over the 8 days was significantly different from zero, and this decline became statistically significant after day 6. Based on this observation, a generalized linear model was developed with a knot at day 6. We centered the time at day 6, the CD4 cell count was regressed with centered data at day 6, and two time intervals were defined: one comprising the days before day 6, and the other including the days after day 6. Therefore, we were able to estimate the mean CD4 cell count on day 6, the rate of decline of CD4 cell counts within the first 6 days, and the rate of decline thereafter.

The overall rate of decline in CD4 cell counts in ST was 6 cells/day (95% CI, 9.02 to 3.62 cells/day; P = 0.000). When we compared the CD4 cell counts in ST at different time points with those in ST at day 6 and adjusted for the baseline K3 EDTA CD4 cell counts, the rates of decline in CD4 cell counts were 3 cells/day (95% CI, 7.4 to 0.48 cells/day; P = 0.085) in the first 6 days and 10 cells/day (95% CI, 24.89 to 4.60 cells/day; P = 0.178) in the subsequent days. The mean total percent change in CD4 cell counts on day 6 compared to the counts on day 0 (baseline) was −8% (where the negative number indicates a decrease), and the amount of change increased substantially thereafter (Fig. 1).

In a subanalysis in which data were stratified by patient ART status, the mean level of change in the CD4 cell count was lower among patients not on ART (−4.9%; 95% CI, −6.9 to −3.1%; P < 0.0001) than among those on ART, but no significant difference among patients on ART was observed (−0.61%; 95% CI, −3.6 to 2.3%; P = 0.685). These results suggest a decline in the CD4 cell counts if whole-blood samples from patients not on ART are tested beyond 5 days postbleeding, although they show no significant difference in the rates of CD4 cell count decline before and after day 5 if patients are already on ART.

In our rural sub-Saharan African setting, we have conducted a comprehensive laboratory and statistical analysis of the performance of ST over time and compared to that of K3 EDTA tubes to determine the utility of ST to improve CD4 cell count accuracy for HIV-positive patients receiving ART. ST have provided a means for maintaining accurate absolute CD4 cell counts over 6 days, and results from these tubes strongly agree with standard K3 EDTA tube performance in our setting. The availability of this technology to prolong the opportunity for accurate and reproducible measurements of CD4 cells and reduce the need for the retesting of specimens is a great benefit for resource-poor settings where climactic conditions and delayed-sample-handling factors can affect the quality of blood samples.

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