Evaluation of TruCount Absolute-Count Tubes for Determining CD4 and CD8 Cell Numbers in Human Immunodeficiency Virus-Positive Adults

CAROL T. SCHNIZLEIN-BICK,1* JOHN SPRITZLER,2 CYNTHIA L. WILKENING,2 JANET K. A. NICHOLSON,3 MAURICE R. G. O’GORMAN,4 SITE INVESTIGATORS,† AND THE NIAID DAIDS NEW TECHNOLOGIES EVALUATION GROUP‡

Department of Medicine/Infectious Diseases, Indiana University School of Medicine, Indianapolis, Indiana 46202; Harvard School of Public Health, Boston, Massachusetts 02146; Division of HIV/AIDS, National Center for Infectious Diseases, Atlanta, Georgia 30333; and The Children’s Memorial Hospital and Department of Pediatrics, Northwestern University Medical School, Chicago, Illinois 60614

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A single-platform technology that uses an internal bead standard and three-color flow cytometry to determine CD4 and CD8 absolute counts was evaluated for reproducibility and agreement. Values obtained using TruCount absolute-count tubes were compared to those obtained using a two-color predicate methodology. Sixty specimens from human immunodeficiency virus type 1-infected donors were shipped to five laboratories. Each site also analyzed replicates of 14 human immunodeficiency virus type 1-infected local specimens at 6 h and again at 24 h. The interlaboratory variability was significantly less with TruCount (median difference in percent coefficient of variation [%CV] between the two methods was −8% and −3% for CD4 and CD8, respectively) than with the predicate method. Intralaboratory variability was smaller, with a median difference in %CV of −1% for both CD4 and CD8 with 6-h samples and −2% and −3% for CD4 and CD8, respectively, with 24-h samples. Use of TruCount for shipped samples resulted in a median CD4 count change of 7 cells (50th estimated percentile) when all laboratories and CD4 strata were combined. For on-site samples, the median CD4 count change was 10 CD4 cells for 6-h samples and 2 CD4 cells for 24-h samples. Individual site biases occurred in both directions and cancelled each other when the data were combined for all laboratories. Thus, the combined data showed a smaller change in median CD4 count than what may have occurred at an individual site. In summary, the use of TruCount decreased both the inter- and intralaboratory variability in determining absolute CD4 and CD8 counts.

Human immunodeficiency virus type 1 (HIV-1) infects cells that express the CD4 receptor (8) and, as a result, depletes its host of CD4 lymphocytes (11). This depletion of CD4 T lymphocytes has been linked to the immunopathogenesis of HIV infection and progression of the disease (9, 13). A CD4 count of ≥200 cells/μl has been included as an AIDS-defining event (5), as these measurements are useful predictors for the onset of opportunistic diseases such as Pneumocystis carinii pneumonia (4). With the advent of highly active antiretroviral therapy, CD4 T-lymphocyte measurements have been used to monitor immune reconstitution (1).

The current predicate methodology for determining absolute CD4 T-lymphocyte counts is dependent upon immunophenotypic identification of cells with fluorescently labeled monoclonal antibodies directed against the CD4 antigen. Relative percentages of CD4 T cells are determined with a flow cytometer. An absolute CD4 count is derived by multiplying the percentage of lymphocytes that are CD3+ CD4+ by the absolute lymphocyte count determined with a hematology instrument. However, the overnight shipment of blood may result in increased intrinsic variability in the absolute lymphocyte count depending on the hematology instrument that is used (10, 16). Therefore, the absolute CD4 count in overnight samples may have increased variability due solely to the hematological determinants.

The need for precise and reproducible monitoring of CD4 T-lymphocyte levels in HIV-infected patients has led several companies to develop simpler methods for measuring absolute CD4 and CD8 T-lymphocyte counts (2, 7, 14, 15, 17). The new single-platform system developed by BD Biosciences (San Jose, Calif.) eliminates the need for multiple technologies (i.e., flow cytometry and hematology) and should be less expensive than predicate methods when labor, cost and inconvenience of repeat samples, and hematology costs are considered. TruCount absolute-count tubes contain a lyophilized pellet that dissolves during sample preparation, releasing a known number of fluorescent beads. By gating the bead population during
in absolute-count bead region [R2]) × [(total no. of absolute-count beads) (test volume [50 µl]).

Hematology measurements. The five hematology laboratories that participated in the study maintained performance that conformed to accepted standards of practice (e.g., College of American Pathologists and National Committee for Clinical Laboratory Standards). All hematologic samples were drawn at the same time as the specimens for flow cytometry. For shipped specimens, the hematologic measurements were performed within 3 h of specimen draw. For on-site specimens, the measurements were performed within 7 h of draw for same-day specimens and within 33 h of draw for the paired 24-h specimen data. White blood cell (WBC) and leukocyte differential (including percent lymphocyte) counts were performed on an automated instrument. If the specimen was rejected or flagged in the lymph node region by the machine, the value was flagged in the database spreadsheet and subsequently eliminated from the study analyses. A cell designated as an atypical lymph or large unstained cell was included in the total lymphocyte number.

Analyses. Criteria for accepting data obtained with SimulSET software included the following: (i) gated lymphocyte purity >85%, (ii) lymphocyte recovery within the gate >90%, and (iii) differences in the CD3 percentages between the CD3+ CD4+ and CD3+ CD8+ tubes ≤7%. Data from individual sites were entered into a spreadsheet designed by the Statistical Data Analysis Center, Harvard School of Public Health, Boston, Mass., and imported into a central database for analyses. Comparisons of the variability of the TruCount method versus the predicate method were based on the Wilcoxon signed-rank test (12) applied to the differences (TruCount method minus predicate method) in the percent coefficient of variation (%CV) of reported CD4 and CD8 counts for each specimen (between-laboratory %CV in the case of the centrally shipped specimens and within-laboratory %CV in the case of local donor specimens). The accuracy of the CD4 and CD8 counts determined by the TruCount method versus the predicate method was tested by the Wilcoxon signed-rank test applied to the differences (TruCount method minus predicate method) in reported CD4 and CD8 counts for each centrally shipped specimen at each laboratory. In the case of CD4 and CD8 counts on replicates from local donors, a log rank test stratified by donor was used. For the primary endpoints, statistical significance was defined as P < 0.05. The primary endpoints for both CD4 and CD8 counts, and combining all CD4 strata, are (i) intralaboratory variability in all laboratories combined using local specimens 24 h old, (ii) interlaboratory variability using centrally shipped specimens, (iii) intralaboratory agreement using local specimens 24 h old, and (iv) interlaboratory agreement using centrally shipped specimens. The P values for the secondary endpoints are exploratory only. Tertiary analyses on the CD4 and CD8 subset percentages were carried out similarly.

RESULTS

Intralaboratory variability for shipped specimens. CD4 and CD8 absolute counts were obtained for 60 specimens shipped to five different laboratories. Statistical analyses of the %CVs of the TruCount method versus the %CVs of the predicate method were performed on the database as a whole, on the CD4 strata, and on individual site data. Table 1 shows that the median %CV for the TruCount method was 8% and 3% less than the predicate method for CD4 and CD8, respectively. When analyzed with regard to CD4 stratum, the <200 cells/µl group showed a significant 23% (CD4) and 9% (CD8) median difference in %CVs for the two methods. These large differences were due to the large median %CVs for the predicate method for samples with a CD4 count of <200 cells/µl. For the remaining two CD4 strata, the median differences between methods were again significantly lower (3 to 7%), favoring the TruCount method.

Intralaboratory variability for 6-h and 24-h replicate samples. Each of the five sites solicited 14 donors whose samples were analyzed as eight replicates at 6 h and eight replicates at 24 h. This therefore, a total of 70 paired samples. 35 with a CD4 count of <200 cells/µl and 35 with a CD4 count of ≥200 cells/µl constituted the database. Table 2 shows that the median difference in %CVs for 6-h replicate samples was −1% (%CV for TruCount − %CV for predicate method) for both CD4 and CD8 counts by both methods. When analyzed with regard to CD4 strata, specimens with CD4 counts of <200 cells/µl showed significant differences in the %CVs for CD4 and CD8 counts (−3 and −1%, respectively). For samples with ≥200 CD4 cells/µl, no differences in median %CVs between
the two methods were seen. Individual site performance reflected what was observed for the database as a whole.

For the on-site replicate samples held overnight, the TruCount tubes generated overall median %CV differences that were less than the predicate method values for both CD4 and CD8 counts (2 and 3%, respectively) (Table 3). When the samples with a CD4 count of <200 cells/µl were analyzed, the median differences in %CVs were 3 and 4%, TruCount values being less than predicate method values for CD4 and CD8 counts, respectively. Samples with a CD4 count of ≥200 cells/µl showed significant median differences with %CVs of −2% for CD4 counts but an insignificant −1% for CD8 counts. In general, individual site performance reflected what was observed for the database as a whole.

**Table 1. Interlaboratory variability for CD4 and CD8 counts for shipped samples**

| CD4 stratum | n | Median CD4 count (cells/µl) | Median %CV absolute CD4 count | Median CD8 count (cells/µl) | Median %CV absolute CD8 count | Median difference in %CVs (TruCount – predicate) |
|-------------|---|-----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------------|
| All         | 60| 315                         | 9                          | 16                          | 988                         | 7                                |
| <200        | 21| 130                         | 11                         | 28                          | 620                         | 6                                |
| 200–500     | 24| 336                         | 8                          | 15                          | 989                         | 7                                |
| >500        | 15| 714                         | 9                          | 10                          | 1,256                       | 10                               |

*P < 0.05, Wilcoxon signed-rank test.

**Table 2. Intralaboratory variability for CD4 and CD8 counts for 6-h replicate samples**

| CD4 stratum | n | Median CD4 count (cells/µl) | Median %CV absolute CD4 count | Median CD8 count (cells/µl) | Median %CV absolute CD8 count | Median difference in %CVs (TruCount – predicate) |
|-------------|---|-----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------------|
| All         | 70| 7                           | 7                          | 7                           | 5                           | 5                                |
| <200        | 35| 8                           | 11                         | 5                           | 6                           | 5                                |
| ≥200        | 35| 6                           | 6                          | 5                           | 5                           | 5                                |

*P < 0.05, Wilcoxon signed-rank test.
analyzed by the same method. Therefore, differences in sample stability were determined separately for the predicate and TruCount methods. For CD4 counts, the median of the difference in fresh and day-old samples using TruCount tubes was 2 cells, compared with 4 cells using the predicate method when all laboratories and CD4 strata were combined (Table 7). In other words, TruCount tubes gave slightly higher CD4 values at 6 h than at 24 h, while the predicate method gave slightly lower values at 6 h than at 24 h. Similar differences were observed when the CD4 strata were evaluated. Each laboratory had its individual bias for paired samples at 6 and 24 h. Table 8 shows a similar data profile for 6-h and 24-h paired CD8 counts. When all strata and laboratories were combined, the median of the differences in CD8 counts using TruCount was lower values at 6 h than at 24 h.

### TABLE 3. Intralaboratory variability for CD4 and CD8 counts for 24-h replicate samples

| CD4 stratum or laboratory | n   | Median %CV absolute CD4 count TruCount | Median %CV absolute CD4 count Predicate | Median %CV absolute CD8 count TruCount | Median %CV absolute CD8 count Predicate | Median difference in %CVs (TruCount − predicate) CD4 | Median difference in %CVs (TruCount − predicate) CD8 |
|---------------------------|-----|---------------------------------------|----------------------------------------|-----------------------------------------|----------------------------------------|------------------------------------------------|------------------------------------------------|
| All strata                |     |                                       |                                        |                                         |                                        |                                                 |                                                 |
| <200                      | 35  | 8                                     | 8                                      | 4                                      | 7                                      | −2*                                             | −1                                              |
| ≥200                      | 35  | 6                                     | 6                                      | 5                                      | 6                                      | −2*                                             | 4*                                              |
| Laboratory                |     |                                       |                                        |                                         |                                        |                                                 |                                                 |
| A                         | 14  | 5                                     | 8                                      | 4                                      | 6                                      | −3*                                             | 2*                                              |
| B                         | 14  | 8                                     | 10                                     | 6                                      | 6                                      | −1                                              | 0                                               |
| C                         | 14  | 9                                     | 9                                      | 4                                      | 8                                      | −3*                                             | −5*                                             |
| D                         | 14  | 6                                     | 6                                      | 4                                      | 4                                      | −1                                              | 0                                               |
| E                         | 14  | 7                                     | 10                                     | 4                                      | 9                                      | −4*                                             | −4*                                             |

*, P < 0.05 using the Wilcoxon Signed Rank test.

### TABLE 4. Agreement between shipped-sample CD4 and CD8 counts

| CD4 stratum and laboratory | n   | Agreement CD4 count Predicate | Agreement CD4 count TruCount | Agreement CD8 count Predicate | Agreement CD8 count TruCount | P  |
|----------------------------|-----|-------------------------------|-----------------------------|-------------------------------|-------------------------------|----|
| All strata                 |     |                               |                             |                               |                               |    |
| All labs                   | 411 | 272                           | −67                         | 7                             | 79                           | <0.01 |
| A                          | 84  | 311                           | −103                        | −17                            | 61                           | <0.01 |
| B                          | 74  | 378                           | −135                        | −34                            | 55                           | <0.01 |
| C                          | 79  | 237                           | −16                         | 13                             | 73                           | <0.01 |
| D                          | 86  | 232                           | −24                         | 12                             | 70                           | <0.01 |
| E                          | 88  | 200                           | −25                         | 25                             | 181                          | <0.01 |
| <200                       |     |                               |                             |                               |                               |    |
| All labs                   | 168 | 115                           | −49                         | 2                              | 40                           | <0.01 |
| A                          | 33  | 168                           | −83                         | −16                            | 9                            | <0.01 |
| B                          | 25  | 178                           | −94                         | −14                            | 35                           | <0.01 |
| C                          | 36  | 106                           | −13                         | 10                             | 42                           | <0.02 |
| D                          | 36  | 105                           | −10                         | 7                              | 48                           | <0.01 |
| E                          | 38  | 103                           | −24                         | 11                             | 55                           | <0.01 |
| 200–500                    |     |                               |                             |                               |                               |    |
| All labs                   | 156 | 337                           | −77                         | 17                             | 101                          | <0.01 |
| A                          | 33  | 380                           | −115                        | 9                              | 76                           | <0.01 |
| B                          | 31  | 396                           | −169                        | −46                            | 62                           | <0.01 |
| C                          | 27  | 318                           | −28                         | 14                             | 83                           | <0.01 |
| D                          | 33  | 319                           | −21                         | 21                             | 83                           | <0.01 |
| E                          | 32  | 274                           | −239                        | 68                             | 225                          | <0.01 |
| >500                       |     |                               |                             |                               |                               |    |
| All labs                   | 87  | 680                           | −102                        | 12                             | 144                          | <0.01 |
| A                          | 18  | 742                           | −145                        | −29                            | 81                           | <0.01 |
| B                          | 18  | 752                           | −182                        | −49                            | 155                          | <0.01 |
| C                          | 16  | 677                           | −26                         | 47                             | 166                          | <0.01 |
| D                          | 17  | 622                           | −49                         | 12                             | 141                          | <0.01 |
| E                          | 18  | 602                           | −14                         | 74                             | 262                          | <0.01 |

* Agreement was determined by subtracting the absolute count obtained by the predicate method from the absolute count obtained by the TruCount method for the same sample. The estimated 10th, 50th, and 90th percentiles of the differences in absolute counts (cells per microliter) are given.

b P value for the estimated 50th percentile by the Wilcoxon signed-rank test.
higher for fresh specimens (15 cells) but lower for day-old specimens (35 cells). Similar individual site biases were observed for CD8 counts for the paired samples at both time points. In general, the stability data showed that use of TruCount tubes resulted in higher CD4 and CD8 counts at 6 h than at 24 h.

DISCUSSION

The current method for the measurement of CD4 absolute counts is expensive and time-consuming and requires multiple manipulations. In addition, most laboratories require separate tubes of blood for the flow cytometry and hematology measurements. Since the hematology measurement is often sensitive to small changes in the blood components, the intrinsic variability of this measurement is especially difficult to minimize. Since the interlaboratory variability in CD4 absolute count for shipped specimens has been unacceptably large, the values for overnight samples may not be reliable. Another confounding factor is that different hematology instruments may have biases toward higher or lower lymphocyte counts (3, 18). This poses a serious problem for patients on HIV-1 intervention protocols if they change where their laboratory CD4 determinations are made. The high variability between any two laboratories may make longitudinal comparisons of CD4 counts inaccurate. The availability of single-platform technologies, which determine CD4 or CD8 absolute counts using only flow cytometry, would decrease this problem and make absolute counts between institutions less variable.

In the current study, which compared single-platform TruCount tubes for CD4 and CD8 counts at 6 h and 24 h, median %CVs were calculated as TruCount %CV − predicate %CV. * P < 0.05, Wilcoxon signed-rank test.

TABLE 6. Variability of subset percentages for shipped and 24-h on-site replicate samples

| Sample group and CD4 stratum | n  | Median %CV for subset | Median difference in %CVs* |
|-----------------------------|----|-----------------------|---------------------------|
|                             |    | TruCount Predicate    | TruCount Predicate        |
|                             |    | CD4                   | CD8                       |
| Shipped                     |    |                       |                           |
| All                         | 60 | 5 9                   | 2 3                       |
| <200                        | 24 | 4 9                   | 2 3                       |
| 200–500                     | 15 | 4 6                   | 2 3                       |
| Replicates                  |    |                       |                           |
| All                         | 70 | 4 6                   | 2 2                       |
| <200                        | 35 | 7 9                   | 1 2                       |
| ≥200                        | 35 | 3 4                   | 2 2                       |

* Calculated as TruCount %CV − predicate %CV. * P < 0.05, Wilcoxon signed-rank test.
Count tubes with a multiplexed method, both the interlaboratory and intralaboratory variability was significantly less using TruCount tubes for both shipped and on-site replicate samples. At the time that the schema was developed, the predicate method in general use did not use CD45 gating. The purpose of this study was to determine the differences in reproducibility and agreement that would occur if a laboratory switched from their current two-color predicate method to a single-platform method. For shipped samples, the variability between CD4 absolute counts using TruCount tubes was about

### Table 7. Agreement between 24-h and 6-h CD4 counts by method

| CD4 stratum and laboratory | n  | Median CD4 count | Agreement, TruCount | P  | Median CD4 count | Agreement, predicate | P  |
|---------------------------|----|------------------|---------------------|----|------------------|---------------------|----|
|                           |    | 24-h 6-h 10th 50th 90th |                     |    | 24-h 6-h 10th 50th 90th |                     |    |
| All strata                |    |                  |                     |    |                  |                     |    |
| All labs                  | 1,120 | 211 219 | -39 -2 39 | <0.01 | 209 200 | -38 4 81 | <0.01 |
| A                        | 224  | 269 279 | -26 6 56 | <0.01 | 289 254 | -12 25 115 | <0.01 |
| B                        | 224  | 173 183 | -47 -8 42 | <0.01 | 207 210 | -22 20 114 | <0.01 |
| C                        | 224  | 237 224 | -18 1 56 | <0.01 | 190 194 | -45 0 65 | <0.01 |
| D                        | 224  | 213 217 | -42 -3 26 | <0.01 | 179 191 | -80 -12 13 | <0.01 |
| E                        | 224  | 186 192 | -56 -9 10 | <0.01 | 158 177 | -34 0 47 | <0.01 |
| <200                      |    |                  |                     |    |                  |                     |    |
| All labs                  | 560  | 104 108 | -23 -3 15 | <0.01 | 109 103 | -26 0 33 | <0.01 |
| A                        | 80   | 171 173 | -16 4 26 | <0.05 | 152 144 | -12 10 160 | <0.01 |
| B                        | 112  | 115 119 | -29 -7 18 | <0.01 | 136 113 | -19 13 74 | <0.01 |
| C                        | 112  | 60 58 | -13 0 14 | <0.01 | 39 49 | -34 -1 28 | <0.01 |
| D                        | 128  | 109 115 | -22 -2 10 | <0.01 | 90 104 | -30 -5 7 | <0.01 |
| E                        | 128  | 104 115 | -26 -6 2 | <0.01 | 87 96 | -34 -2 19 | <0.01 |
| ≥200                      |    |                  |                     |    |                  |                     |    |
| All labs                  | 560  | 400 387 | -59 -1 62 | <0.01 | 401 370 | -57 18 103 | <0.01 |
| A                        | 144  | 320 312 | -35 8 66 | <0.01 | 361 305 | -15 37 104 | <0.01 |
| B                        | 112  | 401 401 | -72 -11 67 | <0.01 | 498 443 | -25 52 142 | <0.01 |
| C                        | 112  | 418 389 | -31 18 91 | <0.01 | 402 368 | -66 12 98 | <0.01 |
| D                        | 96   | 473 492 | -66 -8 39 | <0.01 | 406 451 | -116 -33 31 | <0.01 |
| E                        | 96   | 269 279 | -82 -23 26 | <0.01 | 262 252 | -34 9 91 | <0.02 |

* a Agreement was determined by subtracting the 6-h absolute count from the 24-h absolute count (cells per microliter) obtained for the same sample by the same method. The estimated 10th, 50th, and 90th percentiles of the differences in absolute counts were determined.

* b P value for the estimated 50th percentile, the Wilcoxon signed-rank test.

### Table 8. Agreement between 24-h and 6-h CD8 counts by method

| CD4 stratum and laboratory | n  | Median CD8 count | Agreement, TruCount | P  | Median CD8 count | Agreement, predicate | P  |
|---------------------------|----|------------------|---------------------|----|------------------|---------------------|----|
|                           |    | 24-h 6-h 10th 50th 90th |                     |    | 24-h 6-h 10th 50th 90th |                     |    |
| All strata                |    |                  |                     |    |                  |                     |    |
| All labs                  | 1,120 | 795 828 | -132 -15 133 | <0.01 | 816 820 | -109 35 221 | <0.01 |
| A                        | 224  | 908 945 | -80 65 211 | <0.01 | 1,051 896 | -10 119 401 | <0.01 |
| B                        | 224  | 970 995 | -154 -43 195 | <0.01 | 1,189 1,065 | -73 99 287 | <0.01 |
| C                        | 224  | 848 842 | -132 -10 104 | <0.01 | 851 827 | -158 42 214 | <0.01 |
| D                        | 224  | 756 774 | -99 -16 47 | <0.01 | 751 810 | -149 -22 68 | <0.01 |
| E                        | 224  | 675 765 | -158 -51 5 | <0.01 | 706 674 | -148 0 92 | <0.01 |
| <200                      |    |                  |                     |    |                  |                     |    |
| All labs                  | 560  | 695 716 | -116 -19 84 | <0.01 | 702 684 | -117 18 213 | <0.01 |
| A                        | 80   | 786 754 | -47 74 202 | <0.01 | 731 604 | -11 113 973 | <0.01 |
| B                        | 112  | 684 752 | -138 -49 106 | <0.01 | 1,136 985 | -78 122 316 | <0.01 |
| C                        | 112  | 628 634 | -173 -21 24 | <0.01 | 764 594 | -183 5 199 | <0.01 |
| D                        | 128  | 624 640 | -96 -11 24 | <0.01 | 650 694 | -116 -22 55 | <0.01 |
| E                        | 128  | 691 765 | -107 -29 13 | <0.01 | 668 694 | -124 -8 78 | <0.01 |
| ≥200                      |    |                  |                     |    |                  |                     |    |
| All labs                  | 560  | 926 945 | -159 -8 180 | <0.01 | 928 913 | -96 54 224 | <0.01 |
| A                        | 144  | 1,048 1,132 | -139 62 216 | <0.01 | 1,226 1,065 | -10 123 320 | <0.01 |
| B                        | 112  | 1,076 1,050 | -204 -31 274 | <0.01 | 1,233 1,104 | -66 73 219 | <0.01 |
| C                        | 112  | 1,012 937 | -87 28 181 | <0.05 | 926 887 | -110 68 281 | <0.01 |
| D                        | 96   | 889 894 | -109 -21 84 | <0.05 | 867 893 | -249 -23 80 | <0.01 |
| E                        | 96   | 664 754 | -293 -79 -10 | <0.01 | 719 661 | -166 12 108 | <0.01 |

* a See Table 7, footnotes a and b.
half that using the predicate method. One could argue that this decrease in variability was largely due to the CD45 gating strategy used in the TruCount method and that this decrease in variability could also be achieved with any multiparameter method that used three-color and CD45 gating. If this were true, the difference in variability observed in the subset percentage data for the two methods should account for the majority of the decreased variability in the absolute-count data. However, an analysis of the CD4 subset percentages showed that only a small portion of the decreased variability could be attributed to the use of CD45 gating. This suggests that the TruCount method, in addition to improving the precision of determining lymphocyte subsets over the predicate method, also eliminated the intrinsic variability contributed by the hematology measurements.

When the difference in absolute counts for the same sample by the two methods was calculated, small differences were detected for the database as a whole. For example, the agreement for the 60 shipped samples was a median CD4 count change of 7 cells. Likewise, for on-site replicate samples, the agreement was 10 CD4 cells for 6-h samples and 2 cells for 24-h replicate samples. However, these values were misleading because individual sites had biases in the absolute counts that varied in both magnitude and direction. Individual site evaluations showed significant changes in absolute-count values between the two methods, but since site subset percentages did not show these directional biases (data not shown), one must conclude that the site’s bias in the absolute-count determinations arose from the site’s hematology instrument. For example, laboratory B used a Roche Helios hematology instrument, and that site’s predicate-method absolute counts were consistently higher than those obtained by the TruCount method determinations compared with the other sites, which used Coulter hematology instruments. Thus, it is important that a site perform a comparative study to determine whether a bias in their reported CD4 and CD8 absolute counts will occur if they switch to a single-platform methodology.

Single-platform technology can be more cost-effective than the predicate method when the cost of the WBC and lymphocyte differential counts, the time saved with reduced sample manipulation, and the cost and inconvenience of redrawing blood are considered. For example, the 1999 BD Biosciences list price for the antibody reagents used in the predicate method Simultest panel cost $32.30 per patient test. In comparison, the TriTEST antibody reagents cost $25.40 per patient test. TruCount tubes add $9.60 to the cost of the TriTEST reagents, to bring the single-platform cost to $35.00 for a patient test. Considering that a WBC and lymphocyte differential determination costs more than $2.70 (TruCount minus Simultest costs) and often more than $9.60 (TruCount minus TriTEST costs), the single-platform determination is more cost-effective than the multiparameter methods for reagents alone. In addition, many flow cytometry laboratories are not able to receive hematology laboratory reports by direct data transmission. Since the hematology results must be received by the flow cytometry laboratory before CD4 and CD8 absolute counts can be calculated, misplaced reports can result in absolute-count reporting delays. Occasionally, the tube of blood for the hematology laboratory is not drawn and the CD4 and CD8 absolute counts cannot be reported for the patient sample. In other circumstances, sample quality is so poor that the hematology instrument cannot perform a reliable lymphocyte differential, and again absolute counts cannot be calculated. When hematology values are unattainable for these various reasons, bead-based, single-platform technology can provide meaningful absolute-count data.

Some single-platform methods do not directly determine lymphocyte percentages and only give CD4 and CD8 absolute counts. Examples include the FACSCount system, the Ortho Cytoron Absolute system, the Zymune CD4/CD8 cell monitoring kit from Zynaxis, Inc. (7), volumetric capillary cytometry from Biometric Imaging, Inc. (15), and the TRAx CD4 test kit from T Cell Diagnostics, Inc. (17). However, TruCount tubes and Flow-Count fluorospheres (Beckman Coulter) provide lymphocyte subset percentages as well as absolute counts. The determination of both of these clinical parameters is important because the lymphocyte subset values are often required for monitoring pediatric HIV-positive populations.

In the present study, the predicate method used forward light scatter versus side scatter in comparison to the TruCount method, which used CD45 lymphocyte gating for sample acquisition and analysis. CD45 lymphocyte gating is also routinely used today by flow cytometric laboratories performing three-color, multiparameter analyses and is not unique to the bead-based, single-platform technology used in this study. De-generative samples that must be analyzed with a gating strategy based on forward light scatter versus side scatter often fail to meet acceptable gating criteria and result in blood specimens having to be retested or redrawn. For example, in order for the 60 shipped samples to be analyzable at all five sites, a total of 90 specimens were actually sent to the laboratories. The majority of extra specimens were needed because of unacceptable gating criteria for the shipped samples by the predicate method. However, for those samples that could not be analyzed with the predicate method, CD4 and CD8 absolute counts were almost always obtained by using CD45 lymphocyte gating. The TruCount method had an additional advantage over a three-color multiparameter method in that it could “rescue” samples for which an absolute count could not be calculated because of invalid or incomplete hematology values.

In summary, the TruCount method gave better reproducibility and agreement for both shipped and on-site replicate samples than a multiparameter predicate method. In addition to being more cost-efficient, valid absolute cell counts for degenerative samples could be consistently obtained by the single-platform method. Therefore, the results of this multisite study support the use of bead-based, single-platform technology for routine clinical assessment of CD4 and CD8 absolute cell counts.

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