

Inter- and Intrainstitutional Evaluation of Automated Volumetric Capillary Cytometry for the Quantitation of CD4- and CD8-Positive T Lymphocytes in the Peripheral Blood of Persons Infected with Human Immunodeficiency Virus

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Received 12 August 1996/Returned for modification 9 September 1996/Accepted 18 November 1996

Volumetric capillary cytometry (VCC) is a new technology that involves the detection and enumeration of dually fluorochrome-labeled cells in a precise volume. We compared the accuracy and precision of VCC with the accuracy and precision of flow cytometry and hematology (F&H) for the measurement of the absolute numbers of CD4 and CD8 T cells in the whole blood of patients infected with human immunodeficiency virus. Five laboratories, each with a different F&H system and certified by the National Institute of Allergy and Infectious Diseases flow cytometry proficiency testing program, were shipped aliquots of the same samples from a central site in addition to procuring samples locally. In general, the VCC technology generated CD4 and CD8 T-cell counts which were lower than those obtained with F&H. Intralaboratory variability of replicate CD4 T-cell determinations was similar for both technologies except in the local samples with CD4 counts less than 200/ μ l, where the VCC variability was higher than the F&H variability. Interlaboratory variability on replicate CD4 T-cell counts made by VCC was significantly less than that when counts were made by F&H. The VCC instrument has automated CD4 and CD8 T-cell enumeration in whole blood and has consolidated the process to a single platform. Its performance in this evaluation indicates that it may represent a viable alternative to F&H for obtaining absolute T-cell subset counts.

CD4 T-cell measurement in peripheral blood is a popular method of monitoring disease progression in human immunodeficiency virus (HIV)-infected persons (e.g., see references 4, 6, and 11). Specific levels of CD4⁺ T cells in peripheral blood of HIV-infected persons are also used for diagnostic subclassification, entry and/or end point criteria in multisite clinical trials, and initiation of prophylactic and antiviral therapy (4, 5, 15, 19). The most common method for the determination of absolute lymphocyte subset values per volume of whole blood requires three procedures: flow cytometry to measure the relative proportion of CD4-positive T lymphocytes and hematology procedures for a complete blood cell count and a differ-

ential determination of the percentage of lymphocytes. Absolute lymphocyte subset values are calculated by multiplying the number of leukocytes by the percentage of lymphocytes (to obtain the absolute lymphocyte count) and then multiplying the absolute lymphocyte count by the proportion of lymphocytes expressing the particular marker(s). Although significant progress has been achieved in the quality control and standardization of flow cytometry and hematology (F&H) CD4 determinations (6, 13, 14, 22, 24), the combination of multiple technologies leads to increased variability and logistical concerns. The procedures involved are relatively complex and require expensive equipment with well-trained technicians. These challenges have led to the development of new technologies for absolute CD4 T-cell measurements (7, 9, 12, 20, 21). Recently, a volumetric capillary cytometry (VCC) system which completely automated both the process of staining and the measurement of the absolute number of T-lymphocyte subsets in whole blood was developed (10). This report is a summary of inter- and intrainstitutional evaluation of the accuracy and precision of the VCC technology compared to those of conventional F&H systems for the enumeration of CD4 and CD8 T lymphocytes in the peripheral blood of HIV-positive individuals.

MATERIALS AND METHODS

Patients and samples. Five National Institute of Allergy and Infectious Diseases (NIAID)-certified flow cytometry laboratories participated in the study, which was designed to evaluate differences in the accuracy and intra- and interlaboratory variability of CD4 and CD8 T-cell measurements performed by VCC compared to the CD4 and CD8 T-cell measurements obtained from F&H.

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TABLE 1. Combination of the different flow cytometers and hematology analyzers used in each of the participating laboratories

Site	Flow cytometer	Monoclonal antibody source	Prepn	Hematology instrumentation
A	FACScan (Becton Dickinson)	Immunotech	Optylyse (Immunotech)	StKR (Coulter)
B	XL (Coulter)	Becton Dickinson	Q Prep (Coulter)	Max M (Coulter)
C	Cytoran Absolute	Ortho	Ortholyse (Ortho)	Celldyn 1600 (Abbott)
D	XL	Coulter	Q Prep	Technicon H2
E	FACscan	Becton Dickinson	FAClyse (Becton Dickinson)	Technicon H2

Peripheral blood samples were obtained only from HIV-infected persons. Approval and informed consent were obtained from all participants. Each laboratory obtained blood from at least 10 local donors to perform 10 replicate VCC and F&H determinations, and 144 additional samples were shipped to each of the laboratories by a central contractor (FAST Systems Inc. [FSI], Gaithersburg, Md.). An aliquot of each sample was shipped to each of the five laboratories. Approximately one-third of the shipped samples were blinded replicates, approximately one-third had CD4 T-cell counts of fewer than 200 cells/ μ l, one-third had counts between 200 and 500, and one-third contained greater than 500 CD4-positive T cells/ μ l. Half of the local donor samples had CD4 counts above 200 cells/ μ l.

CD4 and CD8 T-cell enumeration methods: (i) F&H. There were three different analytical flow cytometers and four different hematology analyzers used in different combinations in each of the five laboratories (Table 1 lists the individual F&H systems). All specimens were stained with two-color monoclonal antibody combinations in a whole-blood lysis procedure according to the guidelines adopted by the AIDS Clinical Trials Group (6). Proper biohazard precautions were observed in the collection and handling of patient blood specimens, which were kept at room temperature (18 to 22°C). Originally the study was designed such that the F&H procedures would be performed within 30 h of specimen draw and within 24 h for the VCC procedure. However, shipping and handling problems led to the actual time limit for both procedures being extended to 33 h. The monoclonal antibody labeling of lymphocytes, lysing of erythrocytes, and flow cytometric analyses were performed according to published guidelines (6) with the following panel of fluorescently labeled monoclonal antibodies: CD45-fluorescein isothiocyanate (FITC)/CD14-phycoerythrin (PE), CD3-FITC/CD4-PE, CD3-FITC/CD8-PE, and the appropriate mouse monoclonal isotype controls. The hematology laboratories were maintained according to accepted standards of practice (e.g., College of American Pathologists and National Committee for Clinical Laboratory Standards). The leukocyte and differential (including percent lymphocyte) counts were performed on automated instruments. Cells designated atypical lymph or large unstained cells were included in the total lymphocyte number. There was one laboratory that did not perform automated counts on a large proportion of the shipped samples. All data from these samples were omitted.

(ii) VCC. VCC is a fully automated cell subset enumeration process which involves sample processing and scanning laser imaging of fluorescein-labeled monoclonal antibody-labeled cells in glass capillaries enclosed within disposable sample cartridges (10). The VCC system that we evaluated (Imagn 2000; Biometric Imaging, Mountain View, Calif.) was specifically designed to enumerate CD4 and CD8 T cells in peripheral blood. The reaction cartridges (4T8; Biometric Imaging Inc.) contain two separate processing lanes with precise quantities of dried, fluorescently labeled monoclonal antibodies: CD3 (Cy5) and CD4 (Cy5.5) in one and CD3 (Cy5) and CD8 (Cy5.5) in the other. The emission wavelength maxima for these fluorochromes are 674 nm for Cy5 and 695 nm for Cy5.5. Except for the addition of the samples to the reaction cartridge and the identification of each sample, the process is completely automated. One hundred microliters of whole blood is added to each well within the reaction cartridge. The antibody staining and loading of the cells into each of the capillaries within the cartridges are then achieved with a series of machine-activated incubation, dilution, mixing, and centrifugal loading steps. When the labeled cells have been loaded into the capillaries, a precise area of each capillary is scanned with 633-nm light emitted from a helium-neon laser. The light is reflected off a vibrating mirror across the width of the capillary and simultaneously stepped along the length of the capillary. Light emitted from the monoclonal antibodies is split with a dichroic mirror and collected into two separate photomultiplier tubes. The samples are not washed, so the unbound antibody in the capillary provides a measure of background fluorescence and controls for the amount of antibody in the reaction cartridge. The labeled cells are imaged as peaks of fluorescence above the background level. Coincident peaks of fluorescence (i.e., positive for both Cy5 and Cy5.5) are identified as CD3⁺ and CD4⁺ (or CD8⁺) cells. The concentration of dually labeled cells in the capillary is multiplied by the dilution factor and reported as the absolute number of CD4- and CD8-positive T cells per microliter of whole blood. During the course of the study, the calibration system for the VCC was reevaluated and improved by the development of an absolute standard. It was established that there was a systematic bias of approximately 14% in the dilution factor. The data in this evaluation were collected by using the old dilution factor and were multiplied by 1.1356 to

represent the dilution factor which is currently incorporated into the manufacture of the instrument. Only the recalculated VCC data were used in the evaluation.

Comparison of conventional F&H and VCC. (i) Accuracy: distribution of the differences between the methods. In this evaluation, F&H was considered the "gold standard" for CD4 and CD8 T-cell measurements. The accuracy of the VCC technology was assessed by measuring the difference between CD4 (or CD8) results obtained by VCC and CD4 results obtained by F&H for the same samples. The differences in the results between the technologies were summarized as the 10th, 50th, and 90th percentiles and analyzed separately for shipped samples versus the local samples for each of the following groups; overall, within each of the CD4 groups, and within each individual laboratory.

(ii) Precision: intralaboratory variability of CD4 measurements by VCC versus F&H. The intralaboratory variability was estimated as the percent coefficient of variation (%CV, calculated as [standard deviation/mean] \times 100) of CD4 T-cell determinations made on replicate samples. The intralaboratory variability of replicate CD4 T-cell determinations made by F&H was compared to the intralaboratory variability of replicate CD4 T-cell determinations made by VCC for the same samples. Each laboratory made 10 replicates from each sample obtained from at least 9 local patients, and each laboratory received blinded replicates of various numbers from the contractor. For analysis the replicates were grouped as blinded versus local donor replicates and then further grouped by CD4 level and by laboratory. The median %CV of the replicate CD4 measurements within each of these groups was estimated for both VCC and F&H. Differences in intralaboratory variability between F&H and VCC results were tested with the Wilcoxon paired sample test (see below).

(iii) Precision: interlaboratory variability of CD4 and CD8 determinations made by F&H versus VCC. Each laboratory was sent an aliquot of each of 144 whole blood samples. VCC and F&H CD4 and CD8 T-cell determinations were made for each sample. The interlaboratory variability was estimated as the %CV of CD4 (and CD8) T-cell determinations made for the aliquots sent to each laboratory. The %CV of CD4 and CD8 measurements was determined for both F&H and VCC methods. The median %CV was then established for the following groups: all samples and samples with fewer than 200 CD4 T cells/ μ l, 200 to 500 CD4 T cells/ μ l, and >500 CD4 T cells/ μ l. Differences in interlaboratory variability between F&H and VCC results in each of these groups were tested with the Wilcoxon paired sample test (see below).

Statistical methods. The main statistical method used was the Wilcoxon paired sample test (also called the signed rank test). Differences between the technologies were considered statistically significant when the two-sided *P* value was less than 0.05. Tenth, fiftieth (median), and ninetieth percentiles were computed from the observed sample rather than from the normal approximation, since the differences between the results obtained from each method often failed a test of normality (either untransformed or transformed by using Tukey's power ladder). Regression analyses were not performed, and correlation coefficients were not calculated in our comparison of these two methods. Although the latter statistical methods have been used in each of the evaluations of new technologies published to date, we feel that they are inappropriate for the comparison of two assays (see Discussion).

RESULTS

Patients and samples. A total of 144 samples obtained from HIV-infected persons were made into aliquots and sent to each of the five laboratories via overnight courier. For various reasons not all of the laboratories were able to obtain results for all of the samples. Each local-donor sample was made into 10 replicates (three laboratories obtained replicate samples from 10 donors, one laboratory obtained replicate samples from 9 donors, and one laboratory obtained replicate samples from 18 donors). Local samples were analyzed by four laboratories on the same day that they were obtained, and one laboratory analyzed the samples the next day. The total number of samples received, the number of blinded replicates shipped, and

TABLE 2. Specimens analyzed

Specimen category	No. of specimens					Total
	At laboratory					
	A	B	C	D	E	
FSI						
Sent to laboratory	144	144	144	144	144	720
Received or assayed >33 h postdraw	0	10	1	0	10	21
Flow cytometry and/or hematology not done	15	4	5	1	3	28
VCC not done	9	9	4	0	10	32
Hematology done by hand	0	45	0	0	0	45
Used in analysis of level and/or within-laboratory variability ^a	120	76	134	143	121	594
With both VCC and F&H results used in analysis of between-laboratory variability	92	0	92	92	92	368
Local donors with replicates of 10 for analysis of level and within-laboratory variability	10	9	10	18	10	57 (×10)

^a The following blinded replicates were used for analysis of within-laboratory variability: 1 donor sample was made into five replicates, 3 donor samples were made into triplicates, and 18 were made into duplicates.

the number not analyzed (and the reasons they weren't analyzed) are shown in Table 2.

Accuracy: distribution of the differences between the methods. Accuracy of the VCC CD4 and CD8 T-cell counts was calculated as the difference between the result obtained by VCC and the results obtained for the same sample by F&H (i.e., F&H - VCC). Negative differences are indicative of higher VCC counts. Overall and within each of the CD4 T-cell categories, the CD4 and CD8 T-cell determinations made by VCC were lower than the results obtained by F&H for both local and shipped samples. The median difference in CD4 T cells/μl of whole blood between F&H and VCC was 8 (i.e., 8 more by F&H than by VCC) in the shipped samples and 13 in the local donor samples. The median difference in CD8 T cells/μl between F&H and VCC was 90 more by F&H in the shipped samples and 80 in the local donor samples. When analyzed in each individual laboratory and by the source of specimens, each laboratory obtained significantly lower CD4 values by VCC than by F&H for the local donor samples. However, two of the five laboratories obtained CD4 results by

VCC that were not significantly different from the F&H CD4 results for the shipped specimens. In fact, in one of the laboratories, the VCC-generated CD4 results were higher (although not significantly) than the F&H results. The CD8 counts were significantly lower by VCC than F&H in all of the laboratories for both shipped and locally obtained samples. The distribution of differences between F&H and VCC expressed as 10th, 50th, and 90th percentiles of the differences is presented in Table 3.

In order to graphically illustrate the results obtained by VCC as compared to F&H, the 144 shipped samples were separated into approximately equal-size groups based on the median count by F&H and presented as side-by-side box plots (i.e., F&H versus VCC). The ranges of CD4 (Fig. 1) and CD8 (Fig. 2) results obtained in each CD⁺ group are presented as the minimum, maximum, and 10th, 50th, and 90th percentiles of the values obtained.

It was observed that the ratios of CD4 determinations between the two methods (i.e., VCC/F&H) were more constant than the differences for each of the CD4 groups (i.e., across

TABLE 3. Accuracy of VCC for the determination of absolute CD4 and CD8 T-cell counts per microliter of whole blood^a

Group	Total no. of specimens		Difference														
	FSI	Local	FSI CD4				Local CD4				FSI CD8			Local CD8			
			10th	50th	90th	P ^b	10th	50th	90th	P	10th	50th	90th	10th	50th	90th	
All	594	570	-32	8	107		-13	13	88		-17	90	353		-29	80	305
CD4 count																	
<200	267	280	-14	3	34	0.0007	-12	4	30		-15	88	291		-43	46	235
200-500	182	180	-42	17	104		-7	32	83		-16	116	386		19	98	250
>500	145	110	-85	42	217		-29	63	207		-27	71	370		-41	189	444
Laboratories																	
A	120	100	-36	3	88	0.04	-7	21	80		-45	66	244		22	94	286
B	76	90	-6	44	179		-17	12	64		12	127	320		-40	26	131
C	134	100	-4	33	201		-1	55	210		69	198	528		81	231	440
D	143	180	-45	-4	35	0.07	-19	8	59		-47	45	190		-57	60	278
E	121	100	-51	4	67	0.09	-18	2	70	0.02	-20	71	340		-35	49	200

^a Accuracy was assessed by measuring the differences in the result obtained by VCC and the result obtained by F&H for the same sample (i.e., F&H CD4 or CD8 minus VCC CD4 or CD8). Median CD4 and CD8 counts were greater when measured by F&H than when measured by VCC in each of the laboratories except for CD4 in laboratory D on the FSI specimens.

^b P values throughout the table are <0.00001 unless otherwise indicated.

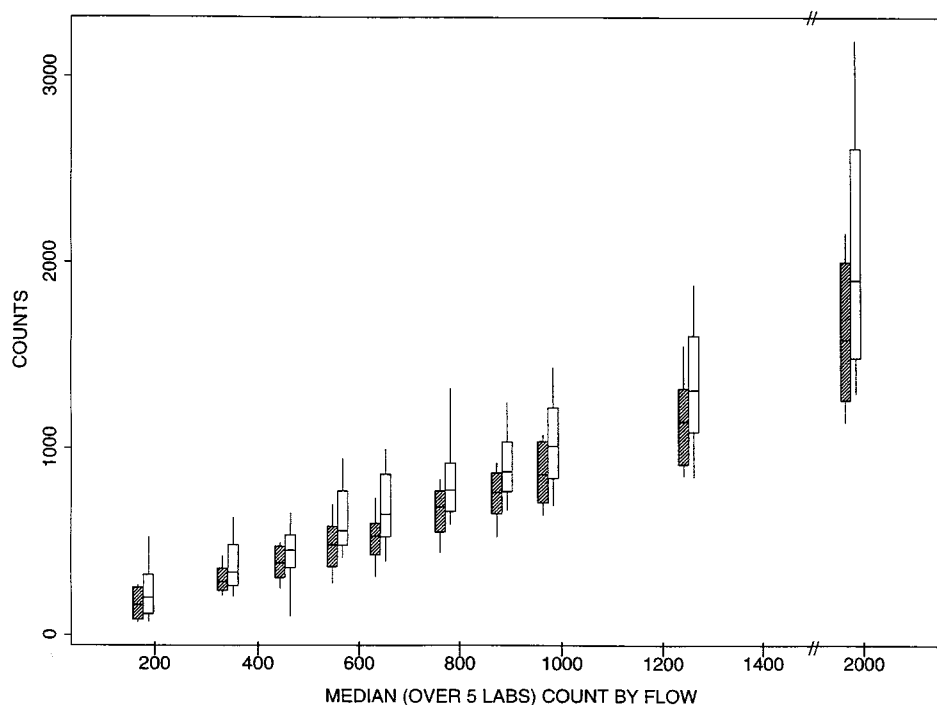


FIG. 1. Range of CD4⁺ T-cell counts obtained by F&H and VCC: box plots of CD4 counts obtained by VCC (shaded) versus CD4 counts obtained by F&H (open boxes). One hundred forty-four whole blood samples from HIV-infected individuals were shipped to each of five laboratories. The median CD4⁺ T-cell result for each sample obtained by F&H was used to form approximately equal-size groups of 14 or 15 datum points (except for the plots closest to 0 CD3⁺ CD4⁺ T cells, which contain 28 datum points). The side-by-side box plots (VCC versus F&H CD4 counts) illustrate the range of results within that group presented as the minimum, maximum, and 10th, 50th, and 90th percentiles.

CD4 levels) as well as for each of the laboratories. The CD4 ratio was 0.94 overall for shipped specimens (0.94 to 0.95 for different CD4 groups and 0.84 to 1.04 for different laboratories) and 0.91 overall for local specimens (0.89 to 0.93 for different CD4 groups and 0.78 to 0.95 for different laboratories).

Precision. (i) Intralaboratory variability of CD4 measurements by VCC versus F&H for blinded replicates (shipped). The %CV of the CD4 and CD8 T-cell determinations obtained by both methods was determined for each replicate sample, and the median %CV was calculated for the following groups: all replicates, the replicates within each laboratory, and the replicates within each of the CD4 categories (see left side of Table 4 for CD4 results [CD8 results not shown]). There were no significant differences between VCC and F&H in the variability (%CV) of replicate CD4 or replicate CD8 determinations in any of these groups.

(ii) Intralaboratory variability of CD4 measurements by VCC versus F&H for unblinded replicates (local donors). All local donor samples were replicates of 10. The intralaboratory variability of the CD4 measurements made by F&H was significantly less than the variability of CD4 determinations made by VCC for the local donor replicates overall, for the CD4 category of fewer than 200 CD4 T cells/ μ l, and in laboratory E (right side of Table 4). The differences in median %CV between the two technologies were not significant in any of the other categories. When analyzed by CD4 group, there were no significant differences observed in the variability of CD8 measurements (data not shown). When analyzed by laboratory, laboratory E had significantly lower variability (%CV) in CD8 determinations made by F&H than in those made by VCC.

(iii) Interlaboratory variability of CD4 and CD8 determinations made by F&H versus VCC. Between-laboratory variability was estimated for a total of 92 samples measured in each of four laboratories (from a total of 144 samples shipped to five laboratories). Overall the between-laboratory variability (median %CV) of both the CD4 and CD8 determinations was significantly less when measured by VCC than when measured by F&H. The difference in %CV for CD4 and CD8 measurements was significant for the samples overall and for each of the CD4 groups except the group with <200 CD4 cells/ μ l ($P = 0.46$). Results are summarized in Table 5.

DISCUSSION

The measurement of the absolute number of CD4-positive T cells in the peripheral blood is a valuable tool for monitoring the immunological status of persons infected with HIV type 1. Additionally, specific CD4 T-cell numbers are included in the criteria for a diagnosis of AIDS, as well as for the institution of antiretroviral and prophylactic therapy. Unfortunately, the current method used to obtain absolute CD4 T-cell counts is expensive and time-consuming and requires multiple procedures, including flow cytometry (to measure the relative number of lymphocytes expressing specific markers) and hematology (to measure the total number of lymphocytes per microliter of whole blood). The cost, intersite variability, and sensitivity to time since blood draw of the hematology measurements have led to the recent development of new procedures to measure the absolute number of CD4-positive T cells from a single platform. VCC is a new technology which has completely automated and unified the process of measuring

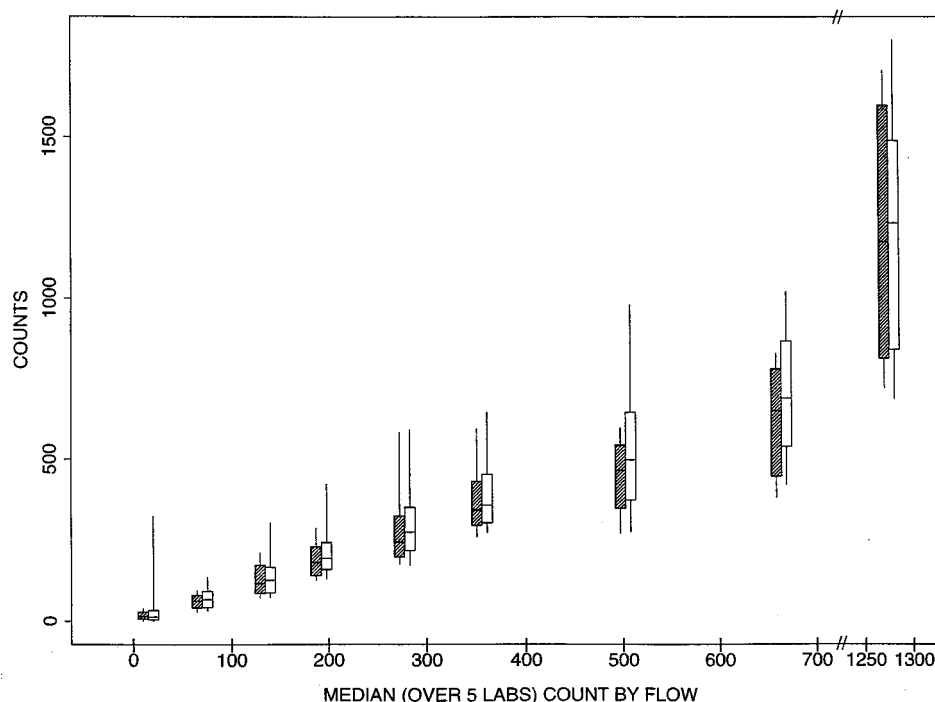


FIG. 2. Range of CD8⁺ T-cell counts obtained by F&H and VCC: box plots of CD3⁺ CD8⁺ counts per microliter obtained by VCC (shaded) versus counts obtained by F&H (open boxes). One hundred forty-four whole blood samples from HIV-infected individuals were shipped to each of five laboratories. The median CD3⁺ CD4⁺ T-cell result for each sample obtained by F&H was used to form approximately equal-size groups of 14 or 15 datum points. The side-by-side box plots (VCC versus F&H counts) illustrate the range of results within that group presented as the minimum, maximum, and 10th, 50th, and 90th percentiles.

absolute CD4 and CD8 T cells in whole blood. This study is an evaluation of the precision and accuracy of VCC CD4 and CD8 T-cell measurements compared to F&H in five NIAID-certified flow cytometry laboratories.

We evaluated peripheral blood only from HIV-infected persons in this study. Compared to F&H, the currently accepted gold standard, VCC measurements resulted in CD4 T-cell numbers that were lower overall (a median of 8 fewer CD4 cells in the shipped samples and 13 fewer in the local samples) than the CD4 T-cell numbers generated by F&H. The differ-

ences in the CD8 results between the two technologies were larger than the differences in the CD4 results. One reason may be that the CD3-positive T cells which express low levels of the CD8 molecule are not counted as CD8-positive T cells by the VCC instrument. The consequences of not counting them remains to be determined, as the significance of this subset (i.e., CD3⁺ CD8^{dim+}) in HIV-infected individuals is not known.

Unfortunately, it is very difficult to compare our results to those of other published studies (7, 9, 12, 16, 18, 20, 21), as all but one (16) have used correlation coefficients and/or regres-

TABLE 4. Within-laboratory variability for CD4 enumeration by VCC versus F&H

Group	Result for:									
	FSI blinded replicates					Local donor replicates				
	Mean CD4 ^a	Median CV by:		F&H CV - VCC CV	<i>P</i> ^b	Mean CD4 ^a	Median CV by:		F&H CV - VCC CV	<i>P</i> ^b
	F&H	VCC				F&H	VCC			
All	459	6.1	6.4	-0.9	0.18	271	7.9	9.7	-1.7	0.0003
CD4 count										
<200	102	8.4	9.8	-2.4	0.10	82	11.1	14.3	-3.6	0.00008
200-500	394	7.8	6.2	-0.3	0.74	348	6.4	7.9	-1.1	0.28
>500	1,016	3.8	3.6	-0.03	0.98	675	5.9	6.7	-0.5	0.76
Laboratories										
A	471	7.9	6.1	2.1	0.26	363	8.3	8.4	-0.1	0.43
B	527	2.0	3.2	-0.05	0.83	250	7.9	8.3	-0.8	0.16
C	531	4.2	6.7	-3.6	0.22	388	8.4	10.9	-1.9	0.27
D	419	5.4	6.0	-0.2	0.29	268	6.8	10.1	-1.4	0.07
E	386	9.0	7.4	-1.9	0.65	185	9.3	18.1	-2.7	0.02

^a CD4 T-cell determination as measured by F&H.

^b Established by the nonparametric Wilcoxon paired sample test (see Materials and Methods).

TABLE 5. Between-laboratory variability of CD4 and CD8 determinations: VCC versus F&H

Group (<i>n</i> ^a)	Interlaboratory variability									
	CD4					CD8				
	Mean CD4 ^b	Median CV by:		Median F&H CV – VCC CV	<i>P</i> ^c	Mean CD8 ^d	Median CV by:		Median F&H CV – VCC CV	<i>P</i> ^c
	F&H	VCC				F&H	VCC			
All (92)	375	14.7	9.2	4.0	0.00002	841	14.1	7.7	6.2	<0.00001
CD4 count										
<200 (39)	85	19.2	18.2	1.7	0.46	695	14.2	9.1	6.4	0.00004
200–500 (30)	342	13.8	8.6	4.1	0.0003	1,020	12.2	8.1	3.3	0.001
>500 (23)	908	11.5	5.5	6.7	<0.00001	855	13.1	6.5	8.3	0.00003

^a Number of samples assayed in each of four laboratories.

^b Mean CD4 level as determined by F&H.

^c Established by the nonparametric Wilcoxon paired sample test (see Materials and Methods).

^d Mean CD8 level for each CD4 group as determined by F&H.

sion lines which are neither practical nor relevant to the problem of comparing two assays (3). We have not calculated a correlation coefficient because it is an inappropriate method to assess whether the results of two techniques agree well (3). In brief, a large correlation coefficient (*r*) does not mean that two assays agree well, since (i) *r* measures the strength of the relationship, not the agreement of two methods (and *r* equals +1 or –1 if the datum points lie on any straight line with a nonzero slope, not only on the line of equality); (ii) *r* is larger if the range of results is larger, and since investigators generally compare two assay methods over the whole range of typical values, “a high correlation is almost guaranteed” (3); (iii) a test of statistical significance based on *r* is irrelevant to the question of agreement, since two assays purporting to measure the same thing are highly unlikely to be as poorly correlated as predicted by the chance agreement of measures of totally different things (e.g., sunspot activity on the day of birth and adult systolic blood pressure); (iv) *r* is markedly influenced by extreme assay values and so is not a good measure of strength of relationship if there are outliers or if data are skewed, i.e., not symmetric about a median (8); (v) there is no standard, clinical or otherwise, by which we can judge if *r* is large enough that one assay method can be replaced by another; and (vi) *r* does not predict how an individual's results would change if one assay were substituted for another. While linear regression would help with item vi if the relationship between the two assays were linear, it would not help with the other five problems. In addition, since %CVs are not constant for the whole range of CD4 counts, a real comparison of VCC and other alternative CD4 methodologies will have to await a study that involves at least some specimens analyzed by several of these assays. A recent report evaluating four different technologies compared the results of each technology with those of F&H by measuring the correlation coefficients as well as measuring the differences between results. The differences were expressed as bias plots (16) and indicated that each of the alternative technologies generated CD4 counts which were lower than the counts generated by F&H. The authors concluded that the difference between F&H and the other technologies was due to the hematology instrument.

Precision, measured as %CV on replicates of the same samples within the same laboratory, i.e., intralaboratory variability, was comparable when samples contained greater than 200 CD4 cells/μl. Within-laboratory variability of CD4 counts for specimens with fewer than 200 CD4 cells was larger when measured by VCC than when measured by F&H. The major

difference in the level of intrasite variability occurred in samples containing CD4 counts of fewer than 50 cells per mm³ (data not shown). This may be because the VCC method counts far fewer events than the flow cytometry method. For count data, since the %CV is equal to constant × (1/square root of the number of events), the smaller sample size means larger variation due to counting error when counts are low with the VCC technology.

The interlaboratory variability of both CD4 and CD8 T-cell counts was significantly less when measured by VCC compared to that when measured by F&H except when CD4 cell counts were under 200/μl. Reducing the number of procedures required to obtain an absolute count is the most likely cause of the reduced variability. It has been reported previously that most of the variability in the determination of absolute lymphocyte subset measurements is contributed by hematology and that different instruments may have a bias toward higher or lower lymphocyte counts (e.g., see references 1, 2, 17, and 23). In this study, the variability of the results obtained from the same samples on the different flow cytometers (i.e., percentage of lymphocytes positive for CD4 or CD8) was less than the variability of the results obtained for the different hematology measurements (i.e., absolute lymphocyte count). These results suggest that a significant amount of the interlaboratory variability of CD4 and CD8 T-cell measurements made by F&H was contributed by the hematology measurements.

The high interlaboratory variability of absolute CD4 T-cell counts by F&H renders the comparison of results between institutions unreliable. For example, a patient may be screened for enrollment into a protocol based on a specific CD4 level at one institution but then be entered into that protocol and monitored for CD4 levels at a different institution. The latter institution may obtain CD4 counts which would have precluded entry of this patient into the protocol in the first place. According to the Centers for Disease Control and Prevention definition for AIDS (2), a patient with a CD4 T-cell count of <200 is diagnosed as having AIDS. Given the interlaboratory variability in absolute CD4 levels between institutions, it is possible that a patient would have AIDS at one institution and not at another. The availability of new technologies which allow absolute lymphocyte subset determinations from a single platform may decrease this problem and make CD4 counts between institutions less variable.

VCC technology has led to the development of a fully automated instrument which measures absolute CD4 and CD8 T-cell counts on a single platform. Assessment of the accuracy

of VCC CD4 measurements with respect to F&H CD4 measurements suggested that the magnitude of the differences between VCC and F&H results was related to the hematology procedures incorporated in the determination of F&H absolute counts. The CD8 T-cell measurements made on the VCC instrument were lower than the measurements made by F&H in each of the laboratories. Advantages of the VCC technology over F&H for the measurement of absolute CD4 and CD8 T-lymphocyte counts include its fully automated operation and its lack of a requirement for highly trained personnel or additional hematology equipment. After the addition of whole blood, no additional hands-on time is required, and CD4 and CD8 T-cell counts from 10 patients can be obtained in one batch. Of potential concern is that VCC is currently unable to provide T-lymphocyte subset percentages. Both the percentage and absolute number of CD4 cells are often monitored in the pediatric HIV-positive populations due to the rapid changes in CD4 absolute numbers observed in infants. However, the importance of obtaining both percentages and absolutes remains an area of considerable debate.

This evaluation suggests that the VCC technology offers a practical alternative to F&H for the determination of absolute CD4 and CD8 T-cell counts in HIV-infected persons. This technology may be particularly well suited for small laboratories, clinics, and hospitals not currently performing flow cytometry as well as for field studies in countries where flow cytometry may not be readily available.

ACKNOWLEDGMENTS

We express our appreciation to Michele Bergeron, Tom Cooley, Virginia Corrochano, Karla Gertis, Tim Postula, and Johnie Robinson for performance of the VCC and F&H procedures. Sincere thanks go to David E. Normansell for critical review of the manuscript and many useful comments.

The study was funded in part by the NIAID and by Biometric Imaging Inc.

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