

Five-Site Evaluation of the Guava EasyCD4™ Assay for the Enumeration of Human CD4+ T cells



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ABSTRACT

Five laboratories evaluated matched, overnight-shipped whole blood specimens using the laboratories' usual (predicate) flow cytometric method and the Guava EasyCD4 assay (in triplicate) for the enumeration of human CD4+ T cells. Between-laboratory coefficients of variability (CV) of the predicate method vs. the EasyCD4 assay for all specimens (n=66), specimens with CD4+ T cell <300 (n=23), ≥300 (n=43), and 250-450 (n=25) cells/uL were, respectively, 11.88% vs. 10.14% (p=0.0089), 16.24% vs. 11.34% (p=0.0006), 9.98% vs. 9.51% (p=0.7627), and 11.73% vs. 11.01% (p=0.7570). The EasyCD4 within-laboratory precision (CV=3.28-5.80%, p=0.0172) was excellent. Agreement between the predicate and EasyCD4 assay was also excellent (R²=0.92). The Guava EasyCD4 assay represents a simple, accurate, and precise alternative to traditional flow cytometric enumeration of human CD4+ T cells.

BACKGROUND

With the recent dramatic increase in access to antiretroviral treatment, simpler and less costly methods to monitor HIV/AIDS treatment — including absolute CD4+ T cell enumeration — are urgently needed. Accordingly, the Guava Technologies, Inc. microcapillary flow cytometric EasyCD4 assay was evaluated vs. the predicate method used in five highly experienced flow cytometry laboratories.

METHODS

Whole blood specimens (n=66) were collected with informed consent by the NIAID Immunology Quality Assurance Laboratory (IQA) and shipped overnight to each of the other four participating laboratories. Each site tested each specimen in triplicate by the EasyCD4 assay; a tube of matched blood was shipped for CD4+ T cell analysis by the usual (predicate) method in each site's licensed Clinical Laboratory. Specimens were stratified by CD4+ T cell count (determined by the usual IQA method), >300 cells/uL (n=23) and ≤300 cells/uL (n=43). In addition, because the CD4+ T cell count range of 250-450 cells/uL is perhaps the most clinically useful range in the developing world, this stratum (n=25) was also analyzed. To estimate between-site variability, %CV were calculated using each site's predicate result and a randomly chosen value from the three EasyCD4 replicates at each site. The Wilcoxon signed rank statistical test was used for between-site comparisons. To estimate within-site variability, %CVs were calculated using the three EasyCD4 replicates and the Friedman statistical test.

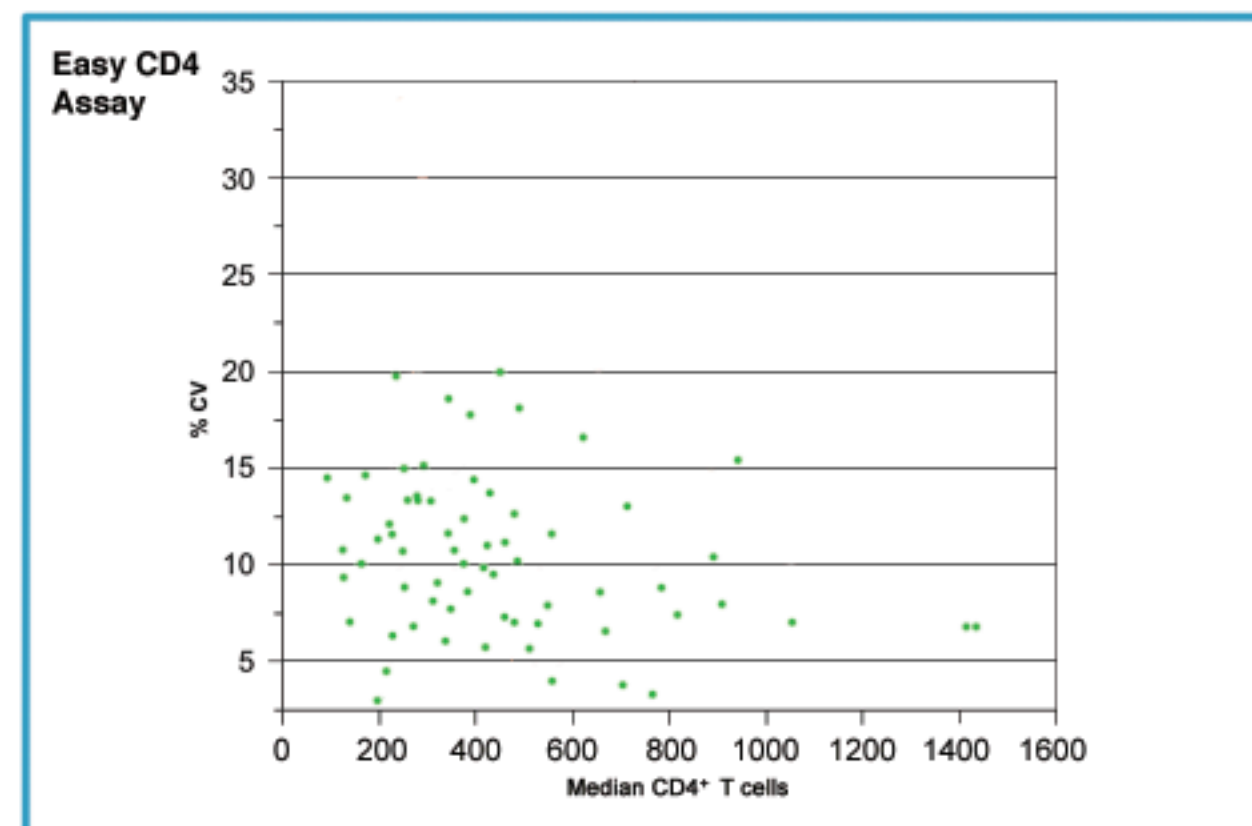
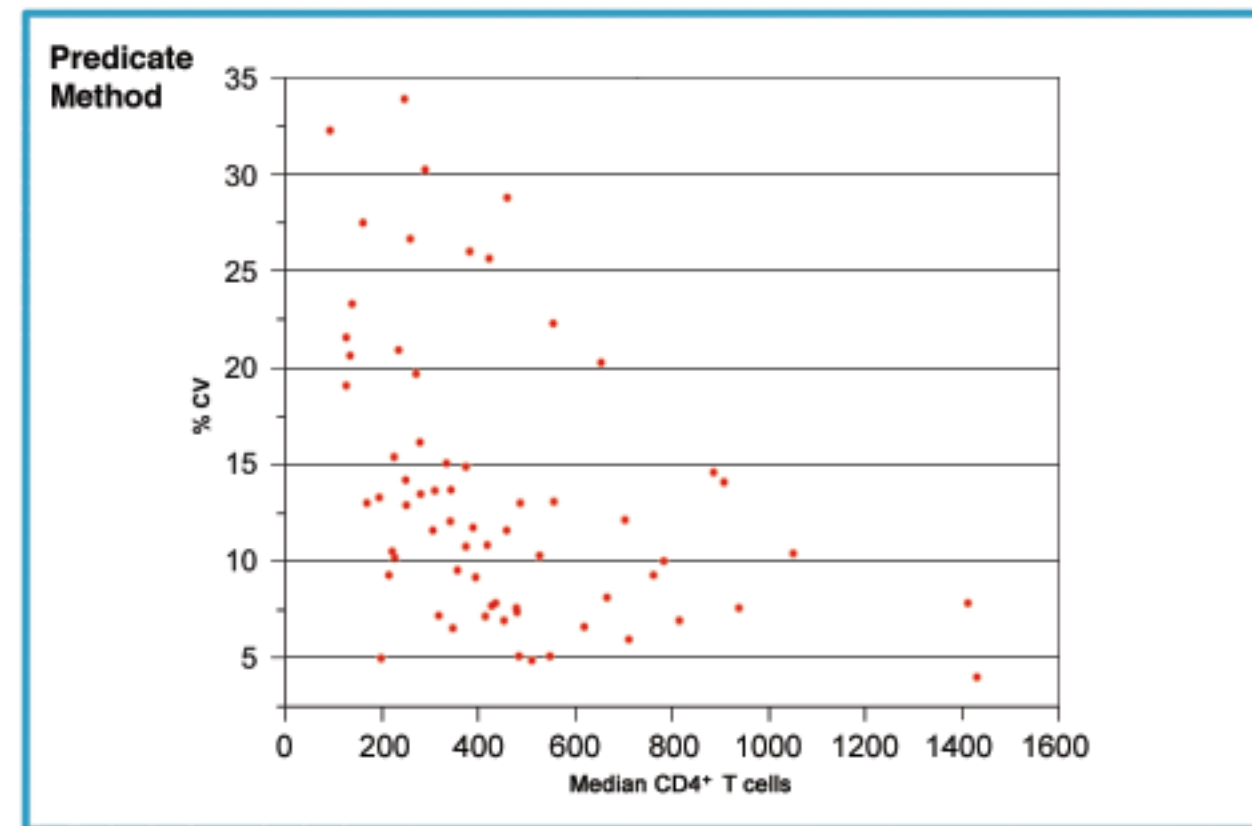


Figure 1. Between-Laboratory Variation. Overnight-shipped whole blood specimens (n=66) were tested in triplicate by the EasyCD4 assay and by the usual (predicate) method in each site's licensed Clinical Laboratory. %CVs were calculated using each site's predicate result (top) and a randomly chosen value from the three EasyCD4 replicates at each site (bottom).

OBJECTIVES

- To compare the between-laboratory variability of CD4+ T cell counts measured by the EasyCD4 assay to the between-laboratory variability of CD4+ T cell counts measured by the laboratories' usual method.
- To estimate the within-laboratory variability of CD4+ T cell counts measured by the EasyCD4 assay.
- To examine the difference between the CD4+ T cell counts obtained by the EasyCD4 assay and the laboratories' usual method.

Table 1. Between-Laboratory Precision

CD4+ T cells Stratum	n	Predicate Median %CV	EasyCD4 Median %CV	p
All Samples	66	11.88	10.14	0.0089
<300 CD4+ cells/uL	23	16.24	11.34	0.0006
≥300 CD4+ cells/uL	43	9.98	9.51	0.7627
250 - 450 CD4+ cells/uL	25	11.73	11.01	0.7570

Wilcoxon Signed Rank Test Asymptotic p values

Table 2. Within-Laboratory Precision

CD4+ T cells Stratum	n	Site1 %CV	Site2 %CV	Site3 %CV	Site4 %CV	Site5 %CV	p
All Samples	66	3.28	4.70	5.06	5.80	5.26	0.0172
<300 CD4+ cells/uL	23	4.99	4.70	6.05	6.94	6.20	0.3875
≥300 CD4+ cells/uL	43	3.22	4.39	4.64	5.18	4.49	0.0459
250 - 450 CD4+ cells/uL	25	5.10	3.74	6.39	7.57	4.94	0.0497

Friedman Test, Asymptotic p values

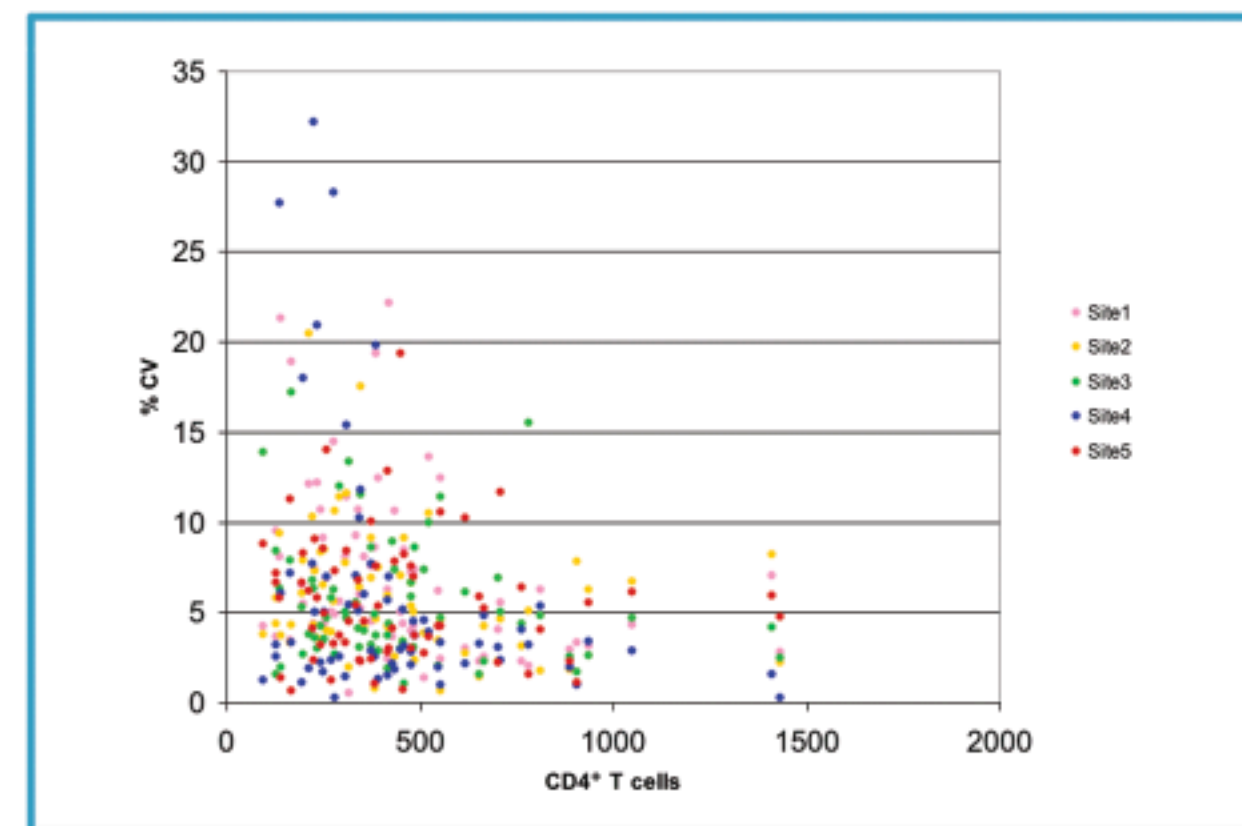


Figure 2. Within-Laboratory Precision. Overnight-shipped whole blood specimens (n=66) were tested in triplicate by the EasyCD4 assay at each of the five laboratories and %CV were calculated for each site.

RESULTS

At all strata evaluated, the **between-site variability** of the predicate method was slightly higher than that of the EasyCD4 method (Table 1, Figure 1), though within the <300 CD4+ cells/uL stratum, the median variability of the predicate method was significantly higher than the median variability of the EasyCD4 method (16.24% vs. 11.34%, p=0.0006).

The EasyCD4 **within-laboratory precision** (Table 2, Figure 2) was also excellent. The CV for all specimens (n=66), specimens with <300 (n=23), >300 (n=43), and 250-450 (n=25) CD4+ T cells/uL were, 3.28-5.80%, 4.99-6.94%, 3.22-5.18%, and 3.74-7.57%, respectively.

Correlation between the predicate method and the EasyCD4 assay (Figure 3) was excellent. The R² values for the predicate method vs. the EasyCD4 assay for each site were 0.91, 0.90, 0.99, 0.93 and 0.94 (mean = 0.93).

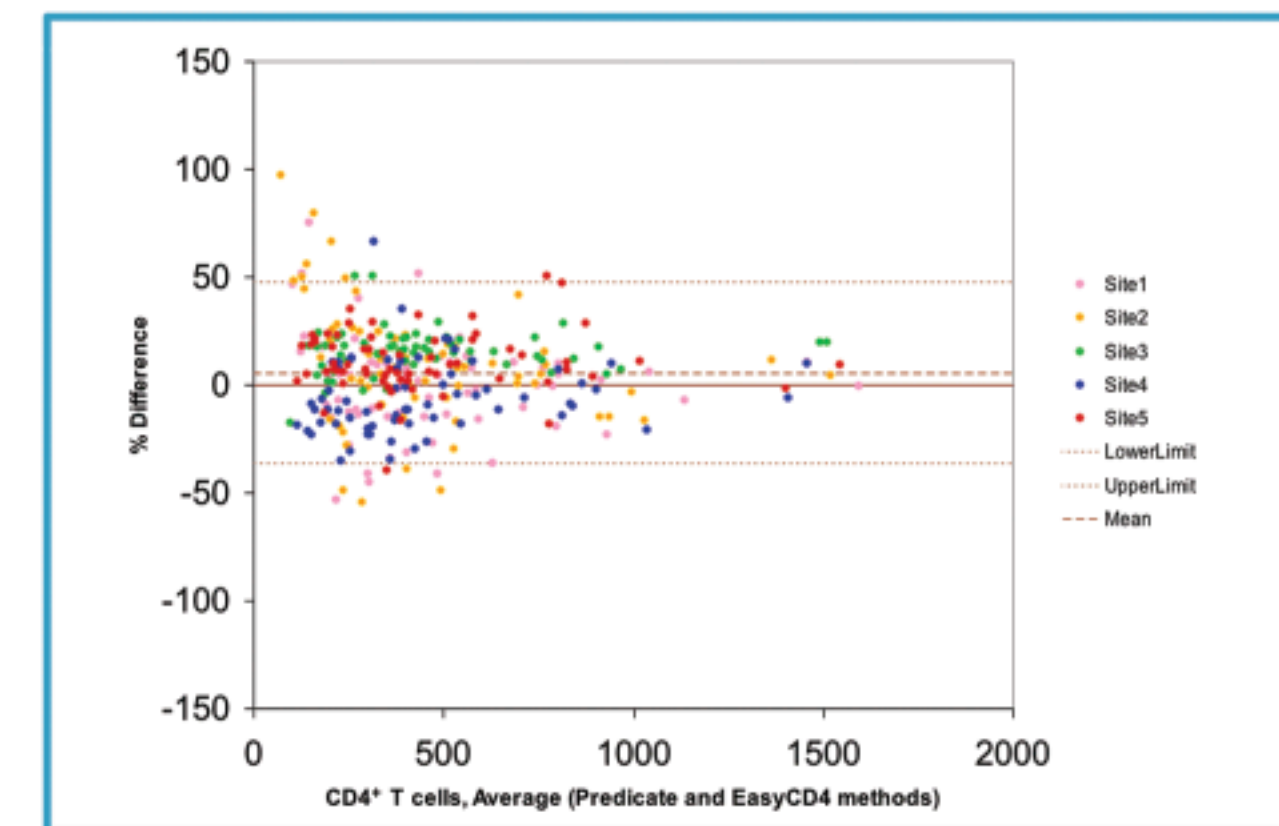
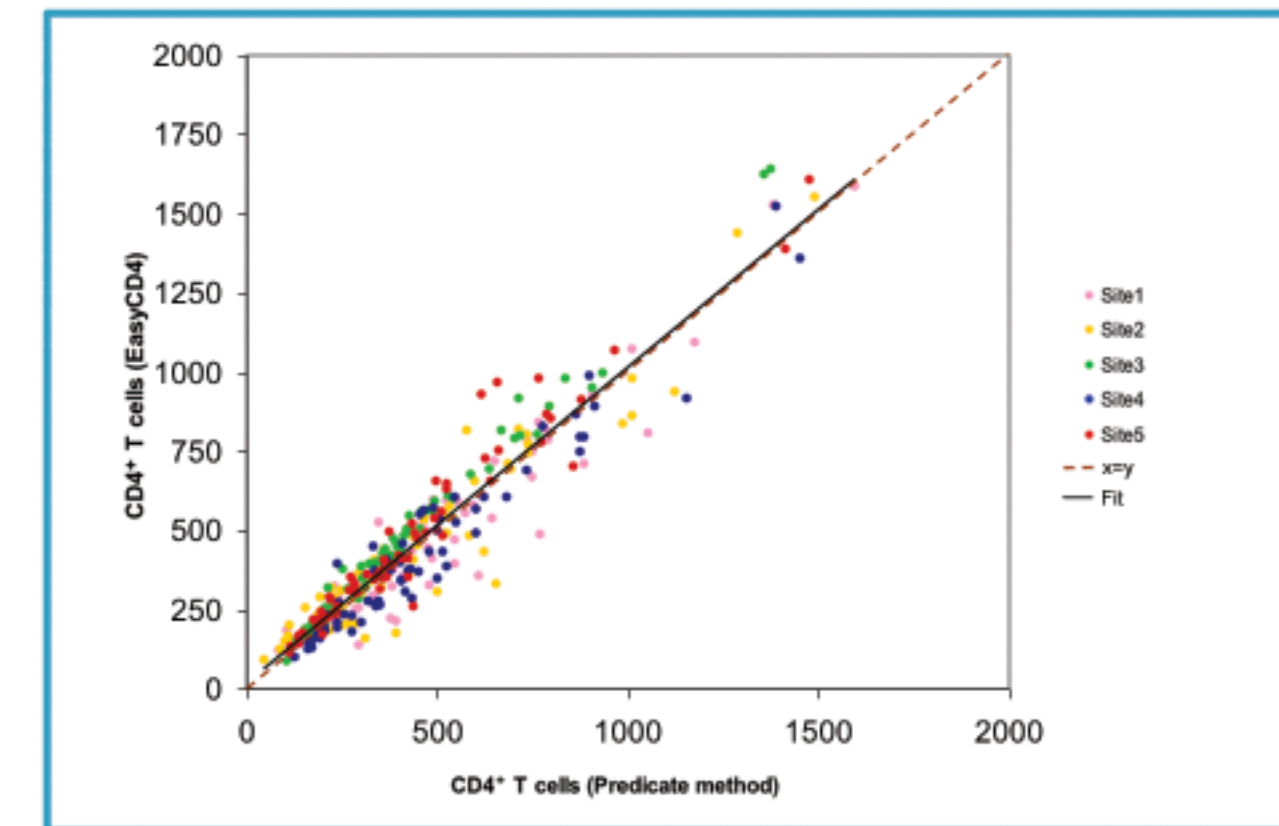


Figure 3. Top: Linear correlation (n=66) between the predicate method and the EasyCD4 assay. The dash line represents x=y. The solid line represents the best fit linear regression for all sites. **Bottom: Percent difference** (n=66) between the predicate method and the EasyCD4 assay. Dashed lines represent the mean (----) and upper and lower (-----) confidence intervals (2 s.d.).

CONCLUSIONS

The EasyCD4 assay, based on microcapillary flow cytometry, correlates very well with the predicate method usually used in five highly-experienced flow cytometry laboratories. Indeed, the between laboratory variability of the EasyCD4 assay was less than that of the predicate methods, especially <300 CD4+ T cells/mL, likely because the EasyCD4 assay is less complicated to perform. The Guava EasyCD4 assay represents a simple, accurate, and precise alternative to traditional flow cytometric enumeration of human CD4+ T cells and should find wide use in both resource-poor and -rich environments.

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