



A Comparison of the LabNow CD4 System to Flow Cytometry in Determining Absolute CD4 Counts, Total Lymphocyte Counts, and CD4 Percent in Whole Blood

The LabNow CD4 System

The LabNow CD4 System was developed for the rapid analysis of key blood measurements in HIV/AIDS patients. This measurement, CD4 lymphocyte count, is a key indicator of disease progression and is used to determine whether drug treatment is initiated or modified. The LabNow prototype system has two components, a disposable microfluidics BioChip and a portable analyzer device. The CD4 BioChip is an integrated assay cartridge consisting of reagents, buffers, and waste storage all within a single use, disposable plastic card. The analyzer device consists of electromechanical actuators, a microscope, digital camera, and output display in an integrated unit. All assay preparation steps have been integrated into the BioChip such that the operator needs only to place a drop of whole blood (approximately 25 to 50 μL) onto the BioChip and place the BioChip into the analyzer. The test is completed automatically in approximately 15 minutes without further operator involvement. The results are displayed for absolute CD4 count, total lymphocyte count, and percent CD4.

Comparison of the LabNow CD4 System to Flow Cytometry

In a recent study conducted at the University of Texas Health Sciences Center, San Antonio (UTHSCSA), whole blood from both immunocompromised (HIV-positive) and healthy (HIV-negative) subjects was analyzed using the LabNow CD4 System and the current gold standard method, flow cytometry. Results were compared between the two assay methods.

After Institutional Review Board approval of the protocol, whole blood from HIV-positive subjects that was submitted to the UTHSCSA flow cytometry laboratory for routine determination of CD4 counts was used for this study. No age limit was set for this cohort of samples. Whole blood from healthy (HIV-negative) subjects, who were at least 18 years old, was also used in the study. For all subjects who participated in the study, any available information regarding demographics, co-infections, and concomitant medications was recorded on electronic data sheets. All HIV-negative subjects who enrolled in the study were required to sign an Informed Consent Form.

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Whole blood specimens were collected into EDTA-containing vacutainer tubes and kept at room temperature until analyzed. Samples were analyzed using the LabNow CD4 System within 8 hours of collection and within the current accepted standard of 48 hours for flow cytometry. Commercially available flow cytometry high and low controls were also analyzed by flow cytometry.

Raw data was collected on electronic data sheets by a laboratory technician. When completed, data sheets were printed, signed and dated by the laboratory technician. The principle investigator was responsible for data collection and maintaining subject confidentiality. Data disks containing updated electronic data sheets and hard copies of the signed electronic data sheets were sent to LabNow once a week during the study.

Data analysis was performed by an independent LabNow consultant who was not involved in data collection. Data analysis included regression to determine correlation between methods and Bland-Altman plots to assess bias between methods. Normality was determined statistically and group differences were determined by t-test and/or analysis of variance (ANOVA).

Analysis Using the LabNow CD4 System

Blood was analyzed with the LabNow CD4 System as follows:

1. Blood (40 μ L) was drawn from the blood collection tube with a pipette.
2. For transfer of the blood to the BioChip, the pipette tip was inserted in the specimen entry port of the BioChip. Transfer of the blood sample to the BioChip was confirmed by visual inspection.
3. The BioChip was inserted into the LabNow Analyzer.
4. Analysis was initiated by simply clicking "OK" on the computer monitor.
5. When the analysis was completed, "Test Complete" was displayed on the computer monitor and the BioChip was ejected from the LabNow Analyzer.
6. Verification was made of the completed test.

Analysis Using Flow Cytometry

Flow cytometry analysis of blood samples and high and low controls were performed using the Becton Dickinson (BD) FACSCalibur Flow Cytometer and BD's IMK reagents per standard laboratory procedures and by following the manufacturer's instructions. Results for CD3, CD4, CD8, CD16+CD56, and CD45 were recorded on the data sheets.

Results

Blood samples were analyzed from all 111 subjects enrolled in this study. Subject age and HIV status are summarized in Table 1. The majority of blood samples were from HIV-positive (75.7%) and male subjects (77.5%). The mean age was higher for HIV-positive subjects than for HIV-negative subjects (49.5 ± 11.1 vs. 34.0 ± 10.7 , respectively). Table 2 summarizes the distribution of blood samples across CD4 subgroups. Most of the specimens (52.2%) had an absolute CD4 count lower than 500 cell counts per μ L.

Table 1: Subject Age and HIV Status

| | HIV-Positive (N = 84) | HIV-Negative (N = 27) | Total (N = 111) |
|---------------|--------------------------|--------------------------|--------------------|
| Gender, n (%) | | | |
| Male | 78 (92.9%) | 8 (29.6%) | 86 (77.5%) |
| Female | 6 (7.1%) | 19 (70.4%) | 25 (22.5%) |
| Age (years) | | | |
| Mean \pm SD | 49.5 \pm 11.1 | 34.0 \pm 10.7 | 48.0 \pm 12.4 |
| Min – Max | 22 – 76 | 23 – 56 | 22 – 76 |

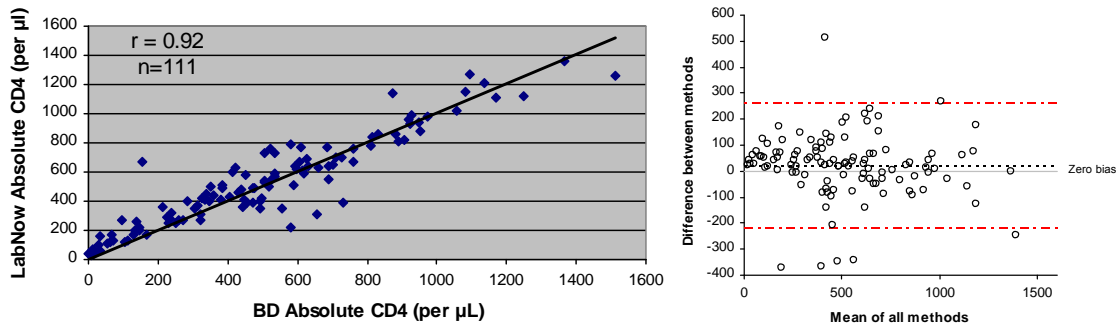
Table 2: Distribution of Blood Samples Across CD4 Subgroups Determined by Flow Cytometry

| CD4 Subgroup (absolute CD4 counts) | Distribution of Samples in Each Subgroup, n (%) |
|---------------------------------------|---|
| 0 – 50 | 7 (6.3%) |
| 51 – 200 | 14 (12.6%) |
| 201 – 350 | 17 (15.3%) |
| 351 – 500 | 20 (18.0%) |
| 501 | 53 (47.8%) |

The correlation coefficient for absolute CD4 counts ($r = 0.92$), total lymphocyte counts ($r = 0.91$), and percent CD4 ($r = 0.94$) indicates a positive correlation between the LabNow System and standard clinical laboratory flow cytometry methods (Figures 1A, 2A, and 3A, respectively).

When the two assay methods were compared with Bland-Altman Plots, the bias for absolute CD4 counts was 25.5 counts (95% CI, 3.5 to 47.6) (Figure 1B), for total lymphocyte counts was 48.4 counts (95% CI, -5.2 to 102) (Figure 2B), and for percent CD4 was 0.07% (95% CI, -0.92% to 1.05%) (Figure 3B). In these figures, the 95% CI is indicated by horizontal dotted lines. The bias remained constant across the three graphs.

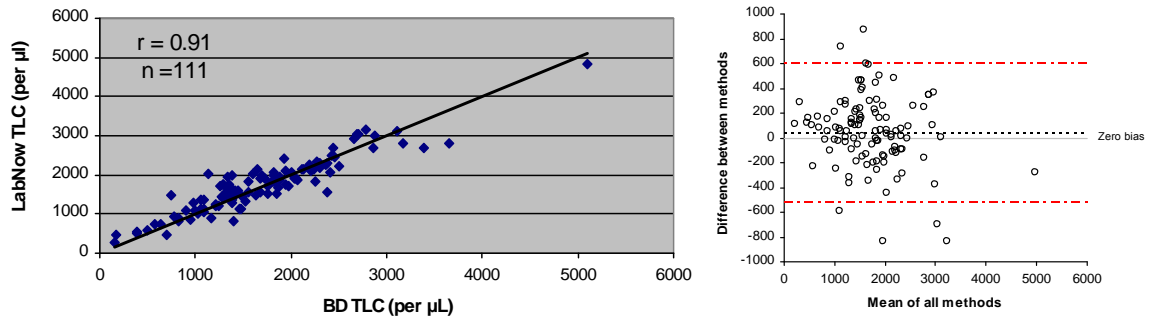
Figure 1: Absolute CD4 Counts



A (left) Correlation Between the LabNow CD4 System and Flow Cytometry

B (right) Bland-Altman Plot Comparing Values Obtained with the LabNow CD4 System and Flow Cytometry

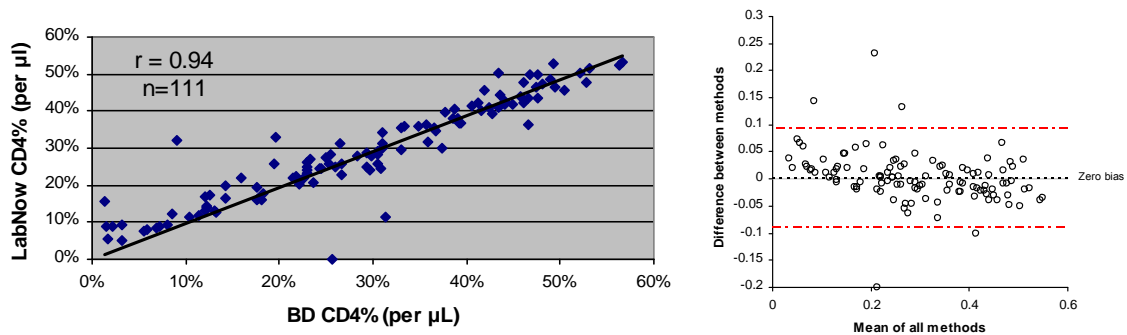
Figure 2: Total Lymphocyte Counts



A (left) Correlation Between the LabNow CD4 System and Flow Cytometry

B (right) Bland-Altman Plot Comparing Values Obtained with the LabNow CD4 System and Flow Cytometry

Figure 3: Percent CD4 Counts



A (left) Correlation Between the LabNow CD4 System and Flow Cytometry

B (right) Bland-Altman Plot Comparing Values Obtained with the LabNow CD4 System and Flow Cytometry

Discussion

Obtaining timely CD4 test results continues to be a major problem for determining treatment of HIV/AIDS patients worldwide. Flow cytometry performed in central reference laboratories is still the standard for providing CD4 counts. The lack of skilled flow cytometry technicians, the high cost of equipment, the need for reliable electrical power, cold chain requirements for reagents, and a long list of other necessary ancillary supplies all limit flow cytometry utilization. In addition, patients often live far from a central laboratory, which requires a significant infrastructure investment to transport blood specimens from remote field clinics to central laboratories for testing. Communicating results back to the clinic and matching them to patients causes further complications and delays. Many CD4 test results can never be matched to the patient in a timely manner to implement the proper care, which causes even further delays and cost to the HIV care system.

The LabNow CD4 System is capable of providing rapid results for absolute CD4 counts, total lymphocyte counts, and CD4% counts. The system has been designed to be easy to use; it requires the user only to place a drop of blood onto the single use, disposable BioChip and then place the BioChip into the Analyzer device. The Analyzer device is lightweight (only 6.5 kg) and can be operated on battery power. These features make it ideal to use in clinical settings at the point of care, even in remote parts of the world. The ability to achieve absolute CD4 counts and CD4% counts in one test allows for the monitoring of both adult and pediatric HIV patients.

In this study at an independent university clinical laboratory site, the LabNow System was compared to a single platform flow cytometry system. Comparative testing demonstrates that the LabNow System provides rapid and accurate CD4 test results with small biases. These results, along with the other advantages of the LabNow System, confirm that a simple to use, portable, point-of-care system can provide accurate CD4 test results and should lead to improved access for patients to CD4 test results in even the most remote areas of the world, and therefore, further improve ongoing care for HIV/AIDS patients.