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BD FACSCount Instrument

A robust and trusted system for measuring absolute
CD4, CD8, and CD3 counts

For in vitro diagnostic use

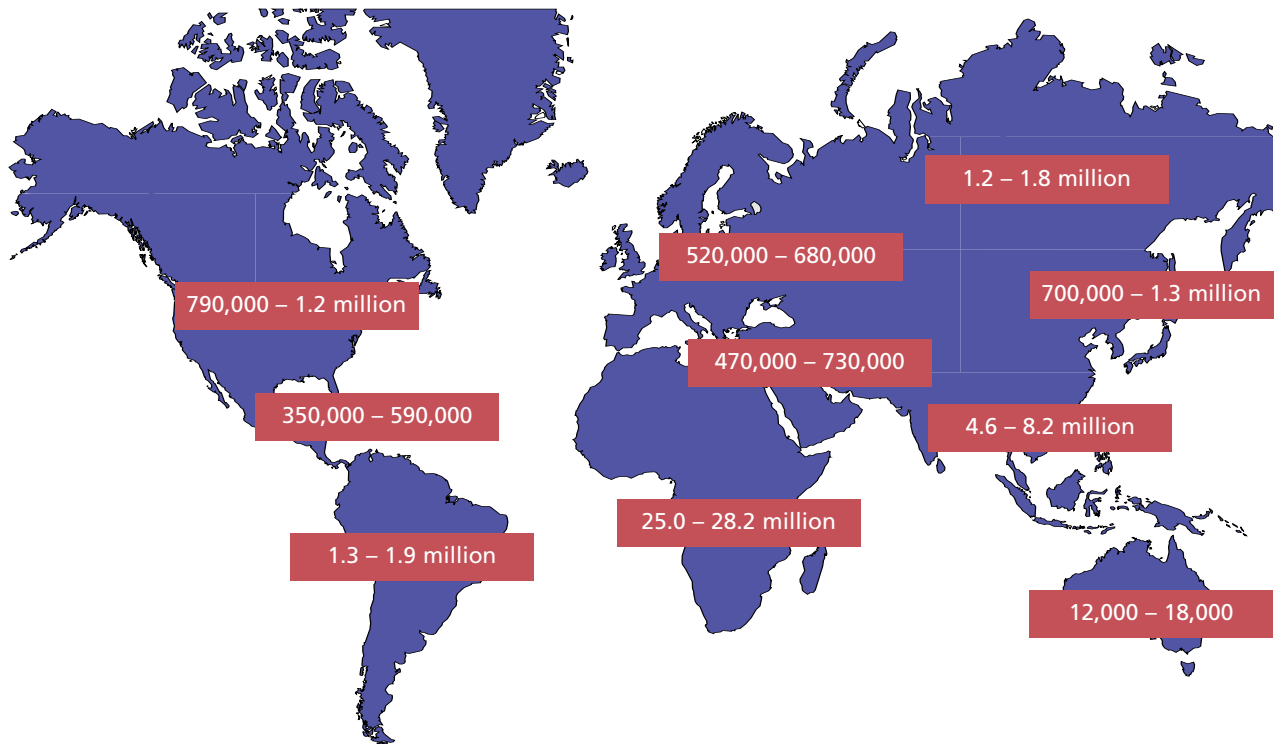
BD Biosciences

Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen



Clinical Significance of CD4

The World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS reported that in 2003, 40 million people around the world were infected and living with HIV, that there were 5 million new infections, and that 3 million people died from AIDS.¹ The continued spread of the disease and the lack of a vaccine or cure make HIV/AIDS one of the greatest challenges of humankind today. Yet there is hope. Appropriate intervention with anti-retroviral therapy and monitoring over the course of the infection has extended and improved life for many people living with this disease. A new, unprecedented level of political will and funding is expanding the number of global initiatives for the prevention, care, treatment, support, and mitigation of the impact of HIV/AIDS.



2003 Estimated Worldwide Distribution of Adults and Children Living with HIV / AIDS¹

Immunophenotyping of T-lymphocyte subsets provides important information for a number of patient conditions. As the primary target of HIV infection, CD4 cells are preferentially infected and depleted with the progression of disease. As the number of CD4+ T lymphocytes decline, the risk and severity of clinical symptoms increase.² For persons infected with HIV, routine monitoring of absolute CD4 counts provides important information on their immune status. Serial measurements of absolute CD4 counts are used as an indicator for initiation of antiretroviral therapy, determination of a patient's response to therapy, and disease progression. Multiple clinical studies of antiretroviral therapies have resulted in a refinement of treatment guidelines for initiation of retroviral therapy for HIV-infected persons. Many factors are taken into consideration: the readiness of the patient for treatment, the prognosis for disease-free survival as determined by absolute CD4 count and viral load, and the assessment of risks and potential benefits associated with initiating therapy. While several indicators are reviewed, it is the absolute CD4 count that carries the greatest weight.³ The World Health Organization developed treatment guidelines for initiating therapy for documented HIV infection in resource-limited settings based simply on the stage of disease and absolute CD4 count or total lymphocyte count.⁴

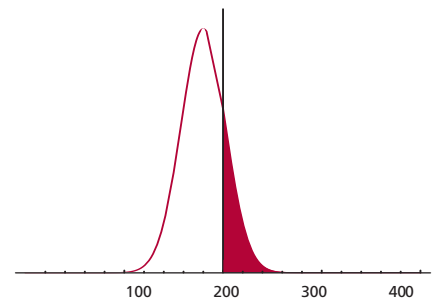
Indications for initiating antiretroviral therapy for HIV-infected patients ⁶			
Clinical Category	CD4+ Count	Viral Load	Recommendations
Symptomatic (AIDS or severe symptoms)	Any value	Any value	Treat
Asymptomatic, AIDS	CD4+ <200/ μ L	Any value	Treat
Asymptomatic	CD4+ >200/ μ L but <350/ μ L	Any value	Offer treatment, although controversial
Asymptomatic	CD4+ >350/ μ L	>55,000 c/mL	Consider therapy or observe
Asymptomatic	CD4+ >350/ μ L	<55,000 c/mL	Defer therapy and observe

WHO recommendations for initiating antiretroviral therapy in adults and adolescents with documented HIV infection in resource-limited settings ⁴
If CD4 testing is available, it is recommended to document baseline CD4 counts and to offer antiretroviral therapy to patients with:
<ul style="list-style-type: none"> • WHO Stage IV disease, irrespective of CD4 cell count • WHO Stage III disease with consideration of using CD4 cell counts <350/μL to assist decision-making • WHO Stage I or II disease with CD4 cell counts <200/μL
If CD4 testing is unavailable, it is recommended to offer antiretroviral therapy to patients with:
<ul style="list-style-type: none"> • WHO Stage IV disease, irrespective of total lymphocyte count • WHO Stage III disease irrespective of the total lymphocyte count • WHO Stage II disease with a total lymphocyte count <1200/μL

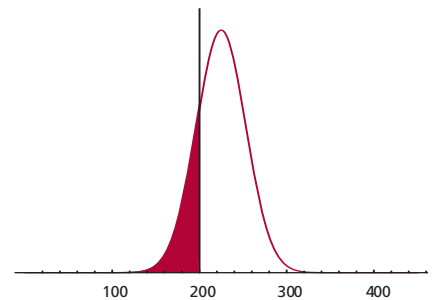
The absolute CD4 count is also considered in the prevention and treatment of opportunistic infections of patients receiving antiretroviral therapy. Susceptibility to opportunistic infections is correlated with the CD4 cell count.⁵ For many pathogens the absolute CD4 count is a critical factor for determining initiation of chemoprophylaxis. In some cases, chemoprophylaxis can be discontinued when restored immunity is indicated and CD4 counts above a specified threshold are reached or sustained.⁵

The graphs to the right illustrate the importance of precise measurements in the determination of patient therapy. Each curve describes the expected distribution surrounding a true CD4 count of 180 and 225. Dual-platform methodology uses absolute lymphocyte counts from a hematology analyzer multiplied by the %CD4+ cells from a flow cytometer to produce absolute counts. Based on reported variation with dual-platform methodology, the probability of misclassification above or below 200 cells/ μ L within a single site is determined by the area under the curve. The probability of misclassifying a patient increases with multiple sites reporting CD4 counts with dual-platform technology.

In the United States, CD4+ T lymphocytes are also incorporated into the case definition of AIDS published by the Centers for Disease Control. The revised classification system, detailed in the December 1992 Morbidity and Mortality Weekly Report, integrates clinical categories with CD4+ T-lymphocyte counts. Precise and reproducible CD4 counts are important to the clear assessment of HIV disease progression.



Patient's true CD4 count: 180
Probability of misclassification by dual-platform technology: 13%



Patient's true CD4 count: 225
Probability of misclassification by dual-platform technology: 19%

AIDS surveillance case definition for adolescents and adults⁷

CD4+ Cell Categories	Clinical Categories		
	A Asymptomatic, acute (primary) HIV or PGL [†]	B Symptomatic, not A or C conditions	C AIDS- indicator conditions
1. ≥500/μL	A1	B1	C1
2. 200–499/μL	A2	B2	C2
3. <200/μL AIDS-indicator T-cell count	A3	B3	C3

The shaded cells illustrate the AIDS surveillance case definition. Persons with AIDS-indicator conditions (Category C) as well as those with CD4+ T-lymphocyte counts <200/μL (Categories A3 or B3) are reported as AIDS cases in the United States and United States Territories.

† PGL=persistent generalized lymphadenopathy. Clinical Category A includes acute (primary HIV) infection.

Research into improving patient care continues and clinical studies are key to determining safety and efficacy. Absolute CD4 counts are an important parameter in many clinical studies to evaluate the efficacy of new and different regimens of therapy. As an example, a recent study measured absolute CD4 counts as an indicator of immune restoration in HIV-positive, antiretroviral-naïve patients receiving the combination of zidovudine/lamivudine plus nelfinavir or nevirapine.⁸ Clinical studies also use CD4 counts as a criterion for determining study groups and study endpoints. For consistent and comparable results across multicenter evaluations, standardization of absolute count determinations is critical.

Precise absolute CD4+ T-lymphocyte values are an accepted and important marker in patient management of HIV infection.

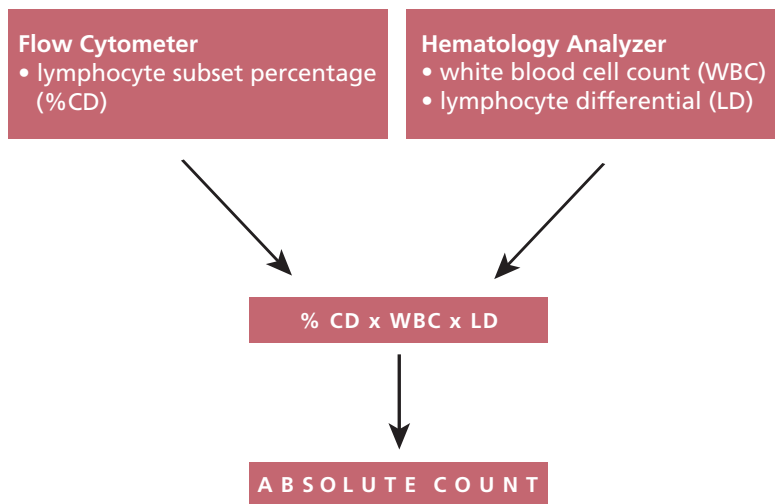
- Physicians treating HIV-infected persons determine the appropriate staging and treatment in conjunction with absolute CD4 counts.
- Persons infected with HIV rely on CD4 counts for information on their immune status.
- Clinical investigators use CD4 counts as criteria for entry into drug trials and as a surrogate marker to measure drug activity and efficacy.
- A precise CD4 count is necessary to accurately describe the epidemiology of HIV and the direction for economic and healthcare policies.

Overview of Dual-Platform and Single-Platform Methodology for CD4 Counting

Subset immunophenotyping by flow cytometry is the most common methodology used in the measurement of absolute CD4 counts in persons infected with HIV. Two techniques exist. In the dual-platform method, absolute CD4 counts are a product of hematology and flow cytometry results. In the single-platform method, absolute CD4 counts are directly measured with flow cytometry.

Dual-Platform Methodology

Absolute CD4 T-lymphocyte count determinations by the dual-platform method require the percentage of T-lymphocytes that are CD4-positive (positive for both CD3 and CD4) from a flow cytometer, and the white blood cell count (WBC) and percentage of white blood cells that are lymphocytes (differential) from a hematology analyzer. The absolute CD4 count is then calculated.



Guidelines and recommendations have helped standardize the practice, but the reliance on two instruments for an absolute count increases the variability of the result. Most of this variability is due to hematology analyzer variability.⁹

As the need for CD4 counts in the clinical laboratory increases, limitations of the dual-platform methodology could prohibit its use. As an example, different instrument platforms have different restrictions on the time in which a sample can be processed. Samples must be run in a timely manner to ensure accurate results. The technical skill required for flow cytometry operators can also be prohibitive to smaller laboratories.

An accurate and reproducible single-platform system is required for the standardization of absolute CD4 count determinations.

Single-Platform Methodology

Absolute CD4 T-lymphocyte count determinations by the single-platform method require measurements from a single tube run on a flow cytometer. The absolute CD4 count is calculated from the measured counts of T-lymphocytes that are CD4-positive (positive for both CD3 and CD4), the measured counts of reference beads, the total number of reference beads in the tube, and the volume of whole blood added.

BD FACSCount™ System Overview

BD FACSCount is a complete system incorporating instrument, reagents, controls, and software. It employs a direct two-color immunofluorescence method for enumerating absolute lymphocyte counts (cells/ μ L whole blood) of the following mature human lymphocyte subsets: CD3+ T-lymphocytes, CD3+CD4+ T-lymphocytes and CD3+CD8+ T-lymphocytes. In addition, the CD4:CD8 ratio is provided. These data can provide important information for staging and monitoring patients infected with HIV.

The BD FACSCount system consists of the following key components:

- BD FACSCount instrument and system software
- Unit test reagent pairs
 - tube of CD4/CD3 reagents and reference beads
 - tube of CD8/CD3 reagents and reference beads
- Fixative solution (5% formaldehyde)
- Control bead solutions (four levels—zero, low, medium, and high)
- BD FACSCount automated pipette (programmed to accurately reverse pipette 50 μ L)

BD FACSCount Reagents

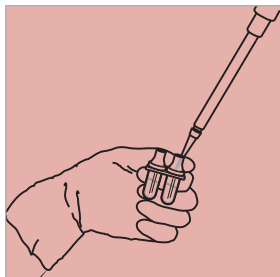
The BD FACSCount reagent kit consists of paired reagent sets containing a mixture of monoclonal antibody reagents conjugated to two fluorochromes and a known number of fluorochrome-integrated polystyrene beads. The first tube in each pair contains CD4 and CD3 antibodies while the second contains CD8 and CD3. The kit also contains formaldehyde fixative (fixative solution).

BD FACSCount Controls

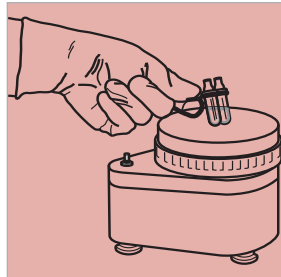
The BD FACSCount control kit consists of paired control bead sets containing fluorochrome-integrated (2- μ m) polystyrene beads at four levels (zero, low [50 beads/ μ L], medium [250 beads/ μ L] and high [1000 beads/ μ L]).

Sample Preparation

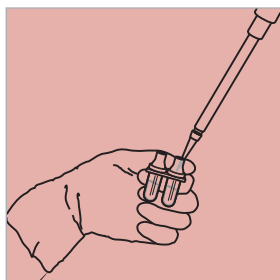
In three easy steps, BD FACSCount samples can be prepared with minimal hands-on time. This reduces the risk of exposure to biohazards and improves laboratory efficiency.



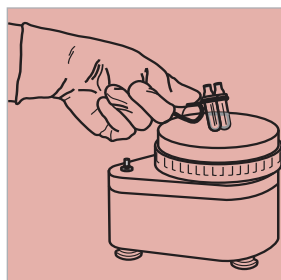
Step 1a
Add 50 μ L blood to each of the two tubes



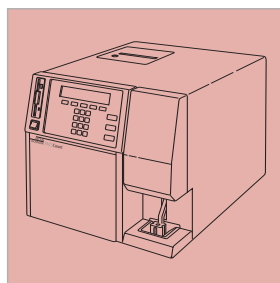
Step 1b
Cap, vortex, and incubate for 60 minutes.



Step 2a
Add 50 μ L fixative to each tube.



Step 2b
Cap and vortex.



Step 3
Run the tubes on the BD FACSCount instrument.

1 Pipette 50 μ L whole blood into each tube, vortex and incubate.

2 Pipette 50 μ L of fixative solution into each tube and vortex.

3 Run on the BD FACSCount instrument.

Control Preparation

1 Pipette 50 μ L of normal whole blood into two pairs of BD FACSCount reagents and prepare as noted on left.

2 Before running on the instrument, pipette 50 μ L of control beads into the corresponding reagent pairs.

3 Vortex and run on the BD FACSCount instrument.

Analysis

BD FACSCount system software provides automated analysis, requiring no operator intervention.

The software provides a report quantifying CD4+, CD8+, and CD3+ T lymphocytes as absolute numbers of lymphocytes per μL (mm^3) of blood, and the CD4+/CD8+ T-lymphocyte ratio. The absolute CD3+ lymphocyte count is reported as the average of the total CD3+ values from the same blood sample stained with the two reagent tubes.

The reportable ranges generated by the BD FACSCount system for each lymphocyte subset are as follows:

CD4	1-2000 cells/ μL
CD8	1-2000 cells/ μL
CD3	1-3500 cells/ μL

Sample Printout

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FACSCount SW Version 1.4 4/02

Sample Results
Reagent Lot ID : 30052121
Reference Bead Counts - beads/ul
CD4 Tube : 1004
CD8 Tube : 2032

Date : 5/15/04 18:51

Last Control Run : PASSED
Control Run Date : 5/15/04 18:42
Control Run Reagent Lot ID : 30052121
Control Run Control Lot ID : 39031721

Accession #:5678

Absolute Counts - cells/ul
CD4 : 838
CD8 : 1107
Total CD3 Average : 2002
CD4/CD8 : 0.76
CD4/CD3 : 0.42
CD8/CD3 : 0.55

Signature: -----
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Comments:-----
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Control Printout

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FACSCount SW Version 1.4 4/02

Control Results
SMITH MEDICAL CENTER
Operator : JKH
Control Bead Lot ID : 39031721
Control Bead Counts - beads/ul
low : 50 med : 247 high : 960
Reagent Lot ID : 30052121
Reference Bead Counts - beads/ul
CD4 Tube : 1004
CD8 Tube : 2032

Date : 5/15/04 18:42

Control Run : PASSED

Lab Normal ID: 12345678

Absolute Counts - cells/ul:
CD4 zero tube : 796
TotalCD3 zero tube : 1927
CD8 low tube : 1113
TotalCD3 low tube : 1993
CD4 medium tube : 799
TotalCD3 medium tube : 1897
CD8 high tube : 1040
TotalCD3 high tube : 1882

Mean Range
Counts -----
CD4 (2 tubes) : 797 3
CD8 (2 tubes) : 1076 73
Total CD3 (4 tubes) : 1924 111
Absolute Counts - beads/ul:
Control Bead zero tube : 0
Control Bead low tube : 56
Control Bead medium tube : 231
Control Bead high tube : 964
Control Bead Results:
R : 0.9998
Slope : 1.00
Intercept : -3
Reference Bead Cluster Locations:
zero tube : 220,211
low tube : 220,212
medium tube : 221,213
high tube : 219,211

Signature: -----
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Comments:-----
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Principles of the Procedure

Staining

The BD FACSCount system is designed to use unlysed whole blood, collected in liquid EDTA. When whole blood is added to the tubes of a sample reagent pair, the fluorochrome-labeled antibodies bind specifically to antigens on the surface of lymphocytes. The BD FACSCount instrument detects two fluorescent colors and measures relative cell size. CD3 cells fluoresce red and CD4 and CD8 cells fluoresce yellow when analyzed on the BD FACSCount instrument. A known number of reference beads is contained in each reagent tube and functions as a fluorescence and quantitation standard for calculating the absolute counts for CD4+, CD8+, and CD3+ T lymphocytes. Fixative solution is added to the stained samples prior to analysis to preserve the integrity of the antibody binding. No lysing is necessary.

System Software and Analysis

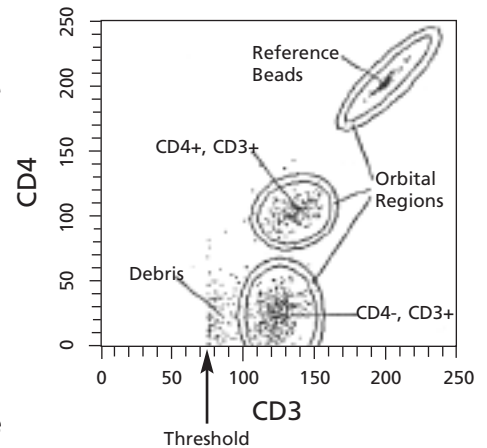
When the sample tubes are run on the system, analysis is automatically completed by the system with no user intervention. The automated analysis is done in five steps. First, the threshold is positioned to eliminate small signal events, such as debris, erythrocytes, platelets, monocytes, and granulocytes, and then a fixed number of events is acquired. Second, the bead and cell populations are located in two dimensions: red versus yellow. Third, ellipses are placed around the cell and bead populations, with the control run providing the starting point. The ellipses optimize their position based on the center of each population. Fourth, an orbital region is placed around each ellipse. Lastly, additional events are collected, up to 30,000 per sample, so that the precision of the measurement is not limited by statistical sampling variation.

In summary, the threshold is positioned, bead and cell populations are located, ellipses are positioned, orbital regions are placed, and data are collected. These steps are performed automatically for both the CD4 and CD8 tubes with no user intervention.

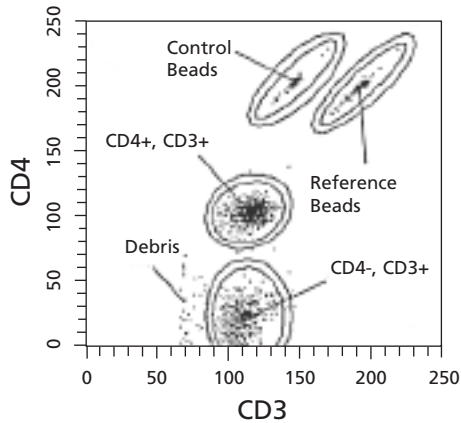
The data is then processed and reported. Absolute counts are determined by a simple ratio:

$$\frac{\text{observed counts from the population of interest}}{\text{observed reference bead counts}} \times \frac{\text{reference bead count}}{\mu\text{L whole blood}} = \text{absolute count (cells}/\mu\text{L) of population of interest}$$

The sample printout reports absolute counts for CD4, CD8, and Total CD3 average. Since there is a CD3 count for each tube, CD3 is reported as the average of the CD3 counts from both tubes.



Graphic representation of the software algorithm



Graphic representation of the control software algorithm

Control Runs

The control run is handled similarly to the sample run, except control beads are added to the prepared normal samples before running on the instrument. BD Biosciences recommends that controls be run at the beginning of each day. The control run determines the counts of four control bead suspensions. During a control run, the software will locate and count the control bead populations in order to verify the linearity of the system. The only difference from the sample run is that an additional ellipse is positioned around the control beads after locating the reference beads. The printout reports the correlation, slope, and intercept for the observed counts versus the expected counts.

The control run also:

- confirms there is staining of the cells
- checks the ranges on the quadruplicate samples of CD3 and the duplicate samples of CD4 and CD8

The results (Pass or Fail) of the control run are reported on each subsequent sample. After running the controls on the system, the data can be recorded on a control chart for that instrument.

Quality Control

The BD FACSCount software has quality control built into its operation so erroneous results are not reported. The highlights of the internal quality control are:

- automatic debris gating: the system finds an optimal gate to eliminate debris from the analysis.
- detection of population overlap: orbital bands are used to ensure each population maintains its integrity and isn't affected by others.
- quality control limits in orbital regions: these regions are used to determine if abnormal distributions of data exist so erroneous information isn't provided.
- count limits on background events to prevent event distributions outside the regions: the data space is defined with unique positions for each of the data clusters. This check looks for undefined data distributions and, if found, prevents reporting of any results.

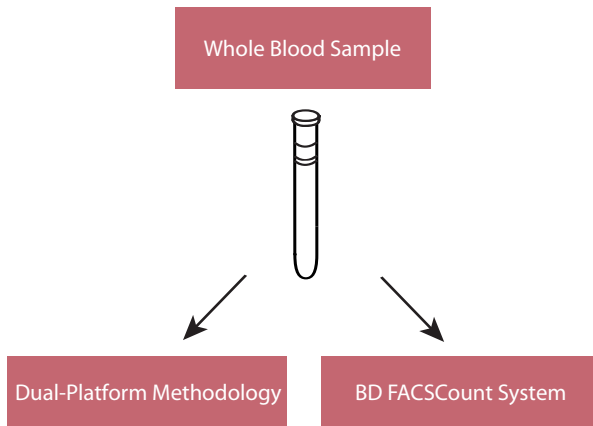
If the data meet the quality limits defined in the software, the software reports results. If the data do not meet the quality limits, the appropriate error messages are printed and sample results (cells/ μ L blood) are not printed.

BD FACSCount Evaluation Clinical Data

Accuracy

Evaluation of system accuracy was determined in comparison to the dual-platform methodology for immunophenotyping. Dual-platform methodology at all sites consisted of a BD FACScan™ flow cytometer along with an available hematology analyzer. The hematology instruments at the clinical trial sites were the Ortho ELT-1500, Coulter STKS, Sysmex NE 8000, and the Cell-Dyn 1600.

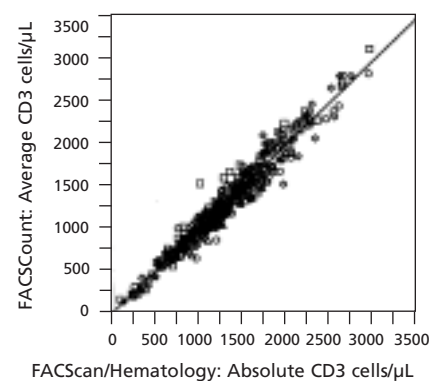
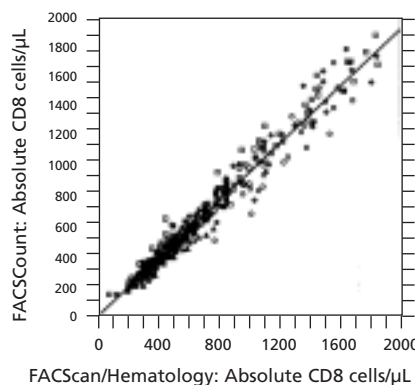
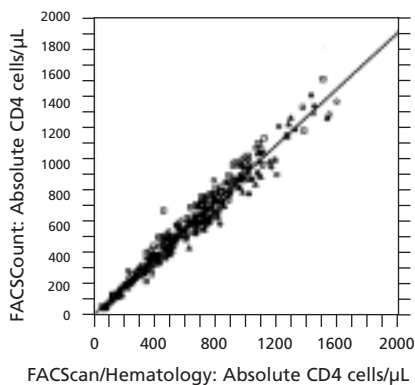
Each whole blood sample was prepared and analyzed in parallel with dual-platform methodology and the BD FACSCount system. Accuracy, for purposes of the studies, was defined as the correlation coefficient (R) between the measurements obtained from the dual-platform methodology and the BD FACSCount system.



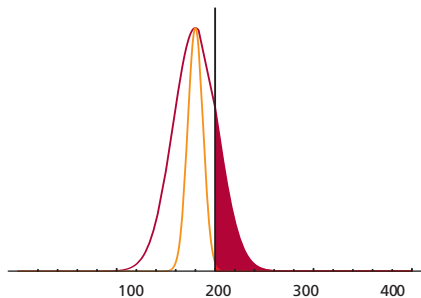
The BD FACSCount system produced results equivalent to the dual-platform method for subset immunophenotyping for absolute CD4, CD8, and CD3 T-lymphocyte counts.

Regression statistics of comparison of absolute counts obtained with the BD FACSCount system versus results calculated by dual-platform method

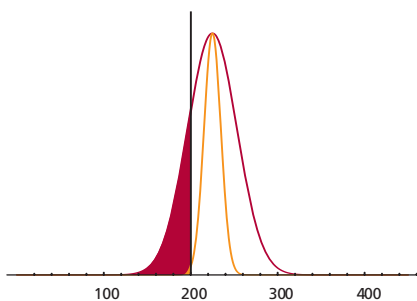
Absolute CD4+ T Lymphocytes	Absolute CD8+ T Lymphocytes	Absolute CD3+ Lymphocytes
n = 351	n = 378	n = 383
R = 0.981	R = 0.983	R = 0.975
slope = 0.982	slope = 0.943	slope = 0.960
intercept = 15.161	intercept = -7.58	intercept = 18.895



Regression graphs for comparison of absolute counts obtained with BD FACSCount system versus results calculated by dual-platform methodology



Patient's true CD4 count: 180
 Probability of misclassification
 by dual-platform technology: 13%
 by BD FACSCount: 0.06%



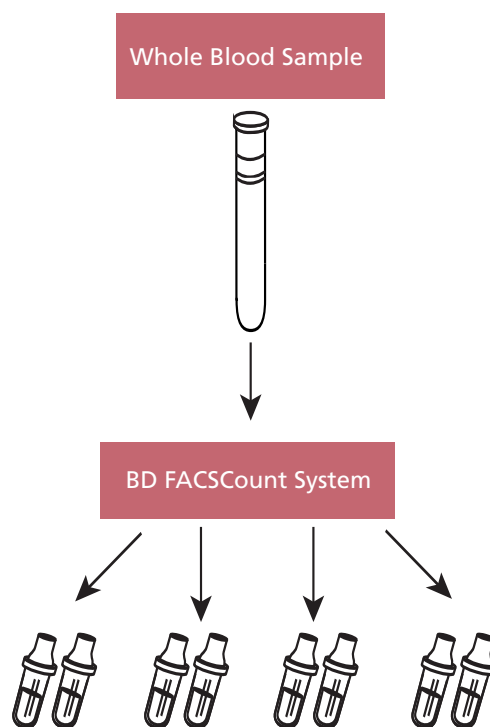
Patient's true CD4 count: 225
 Probability of misclassification
 by dual-platform technology: 19%
 by BD FACSCount: 0.5%

Precision

The sequential monitoring of persons infected with HIV has emphasized the need for reproducible counts. An important aspect of BD FACSCount clinical trials was to estimate the reproducibility of the system with replicate processing of individual samples.

To characterize tube-to-tube reproducibility, four BD FACSCount reagent pairs were stained separately for each sample and acquired on the system. The replicate data were used to calculate coefficients of variation across the reportable range of the system as well as at CD4 values surrounding therapeutic decision points.

The BD FACSCount system showed improved precision over the reported variability in dual-platform methodology.



Coefficients of variation (CVs) determined across the reportable range of BD FACSCount system parameters at two clinical sites

	CVs at Site 1	CVs at Site 2
Absolute CD4+ T lymphocytes		
100–300 cells/ μ L	2.32%	3.38%
400–600 cells/ μ L	3.95%	4.59%
50–2000 cells/ μ L	3.75%	5.10%
Absolute CD8+ T lymphocytes		
100–2000 cells/ μ L	2.99%	4.05%
Absolute CD3+ Lymphocyte		
100–3500 cells/ μ L	2.51%	3.07%

Precision data for CD4 counts less than 50 cells/ μ L are shown in the table below. Blood samples from 10 abnormal subjects were prepared (four times) with BD FACSCount reagents within 24 hours of collection. (No performance characteristics were established for CD3 or CD8 counts below 100.)

CD4+CD3+ Range	Mean* (cells/ μ L)	Subjects	n	SD as an Estimate of Within-Sample Reproducibility†	Upper 95% Confidence Limit on SD§
1-20 cells/ μ L	7.2	6	22	0.78	1.10
21-50 cells/ μ L	35.0	4	16	2.63	4.08

* Mean is the pooled mean, for example, \bar{Y} = the mean of the individual means.

† SD = standard deviation, the pooled standard deviation

$$SD = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + \dots + (n_k - 1)s_k^2}{n_1 + n_2 + \dots + n_k - k}}$$

§ 95% confidence interval for SD of normal distribution

Interlaboratory Reproducibility

As a criterion for patient enrollment in clinical studies, specific levels of absolute CD4 are often used to define study groups. Consistent and comparable reporting of absolute counts becomes critical to the results and the conclusions of the study. As a dedicated system for providing absolute CD4, CD8, and CD3 cell counts, the BD FACSCount system produces comparable results across different laboratories.

Comparability and reproducibility were accomplished by preparation and analysis of whole blood samples in parallel at two different clinical sites. Regression analysis determined the comparability of absolute counts obtained using the BD FACSCount system at both sites. Tube-to-tube reproducibility was also estimated at both sites by four separately stained replicates of each sample. The replicates were used to calculate coefficients of variation across the reportable range of the system for normal controls and abnormal samples separately.

Regression statistics for comparison of absolute counts obtained from the same samples by two different sites

	n	R	slope	intercept
Absolute CD4+ T Lymphocytes	103	0.993	0.984	10.180
Absolute CD8+ T Lymphocytes	124	0.995	1.014	-0.347
Absolute CD3+ Lymphocytes	126	0.995	0.996	21.177

Absolute CD4+ T lymphocytes

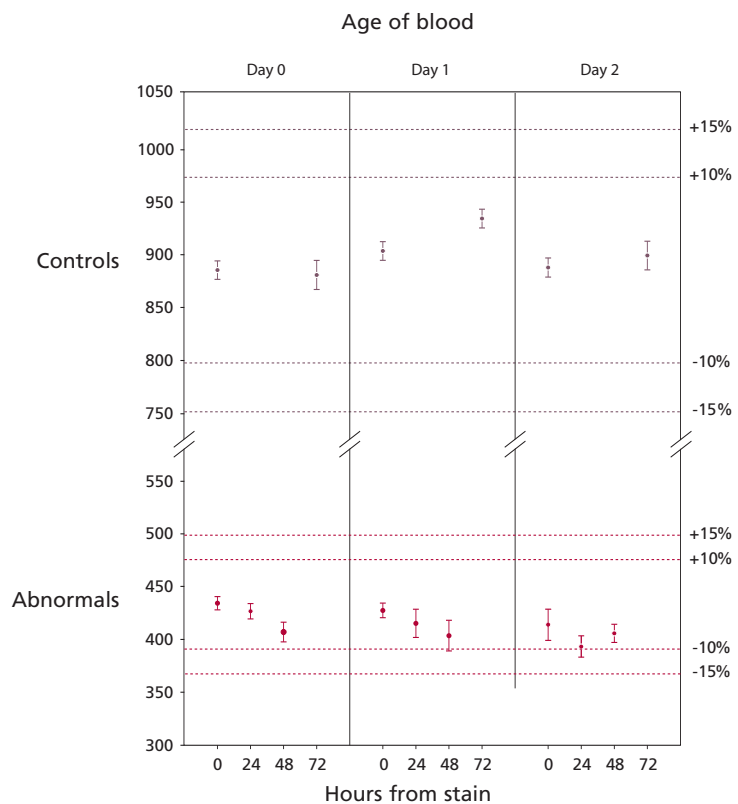
	n	mean	df	SD*	% CV
Controls					
Site 1	34	786.39	49	23.50	2.99
Site 2	34	771.53	78	31.88	4.13
Abnormals					
Site 1	14	292.81	39	12.14	4.15
Site 2	14	288.18	48	14.92	5.18

* SD as an estimate of within-sample reproducibility

Stability

To increase flexibility in sample processing, comprehensive studies evaluated the performance of the BD FACSCount system for extended whole blood and stained sample storage times. Stability was determined by comparing the results from each time point to time zero. Time-zero results were collected immediately following the recommended sample preparation procedure using whole blood less than 6 hours from draw. For these studies, stability was defined as changes equal to or less than 10% of the time-zero value for 24-hour storage times and 15% of the time-zero value for 48-hour storage times

Storage of whole blood produced stable results up to 48 hours from draw when stained and analyzed with the BD FACSCount system. Storage of stained samples produced stable results up to 48 hours following sample fixation with the BD FACSCount system.



Absolute CD4+ T-lymphocyte counts across donors at each time point, including the 95% confidence interval (CI), and % CV												
Age of blood	Day 0				Day 1				Day 2			
Age of stain	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr	0 hr	24 hr	48 hr	72 hr
Controls mean (cells/ μ L)	885	-	-	881	903	-	-	934	887	-	-	898
95% CI	± 8.72	-	-	± 13.75	± 8.92	-	-	± 8.95	± 9.13	-	-	± 13.49
% CV	2.66	-	-	4.22	2.67	-	-	2.59	2.78	-	-	1.05
Abnormals mean (cells/ μ L)	434	426	407	-	427	425	403	-	413	393	405	-
95% CI	± 6.25	± 7.33	± 9.41	-	± 6.87	± 13.34	± 14.52	-	$+14.81$	$+10.15$	$+8.49$	-
% CV	3.29	3.92	5.28	-	3.67	7.34	8.22	-	7.96	5.90	4.56	-

Reference Range

To establish reference ranges for BD FACSCount system parameters, data were collected from three geographically distinct sites on hematologically normal samples. The age criterion for donors in the study was 18 to 65 years. Differences in age, gender, and race were considered in the poolability of the data across sites. Age differences were observed for CD8+ T lymphocytes and ratio values; therefore two reference ranges are presented. Gender differences were observed for CD4+ T-lymphocyte values and two ranges are also presented. No differences ascribed to race were observed in the study.

BD FACSCount system parameter reference ranges					
Parameter	Sex	Age	n	Mean cells/ μ L	95%* range cells/ μ L
CD4+ Lymphocytes [†]	Male	18–65	92	702	355–1213
	Female	18–65	57	798	470–1298
CD8+ T Lymphocytes	Both	18–40	92	433	208–796
	Both	41–65	58	346	144–699
Average CD3+ T Lymphocytes	Both	18–65	151	1206	688–1955
CD4:CD8 Ratio [§]	Both	18–40	92 [†]	1.87	0.92–3.41
	Both	41–65	58	2.49	0.83–6.10

* The 95% range is obtained by fitting an appropriate distribution to the data and then calculating the central 95% area of the fitted distribution.

[†] Two did not have gender designation and were omitted.

[§] One subject had a missing value for CD8. Cells/ μ L is not applicable to the CD4:CD8 ratio.

Conclusion

The BD FACSCount system is a unique and complete system of software analysis, unit dose reagents, and four levels of bead controls to provide absolute counts. Because it is a complete system it eliminates the need for an external hematology result. This change removes the variability found in absolute counts, which had been introduced by combining flow cytometry results with those from a variety of automated cell counters.¹⁰ With dual-platform methodology, absolute count values for lymphocyte subsets might not always be comparable across laboratories. The BD FACSCount system reduces interlaboratory variability.

As a complete system, BD FACSCount precisely measures absolute numbers of CD4+ T lymphocytes, the cellular parameter most closely associated with HIV disease progression and therapy decisions. Even with low CD4 counts, the BD FACSCount system gives consistent, accurate results. In addition, the system measures the absolute numbers of CD8+ and CD3+ T lymphocytes and the CD4:CD8 ratio.

Today it is imperative that clinicians have cost-effective, reliable tools to monitor the immune status of patients, especially those infected with HIV. Clinicians utilize absolute CD4 T-lymphocyte counts in the staging and monitoring of disease progression and in making therapeutic decisions. The BD FACSCount system, which is dedicated to providing information used to monitor immune status, meets all these requirements.

References

1. Joint United Nations Programme on HIV/AIDS, World Health Organization. AIDS epidemic update. December 2003. Available at: <http://www.who.int/hiv/pub/epidemiology/epi2003/en/>
2. Amman AJ, Abrams D, Conant MA, et al. Acquired immune dysfunction in homosexual men: immunologic profiles. *Clin Immunol Immunopathol.* 1983;27:315-25.
3. Dybul M, Gulick RM. State of the Art: The new DHHS HIV treatment guidelines and current controversies in antiretroviral therapy. *PRN Noteb.* 2003; 8:15-24.
4. World Health Organization. Scaling up antiretroviral therapy in resource-limited settings: treatment guidelines for a public health approach. 2004 Revision. Available at: http://www.who.int/3by5/publications/documents/arv_guidelines/en/
5. 2001 United States Public Health Service (USPHS) / Infectious Disease Society of America (IDSA). Guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus. November 28, 2001. Available at: <http://www.aidsinfo.nih.gov/>
6. The Department of Health and Human Services (DHHS). Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents: the living document. March 23, 2004. Available at: <http://www.aidsinfo.nih.gov/guidelines/>
7. 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults. *MMWR.* 1992;41:961-962.
8. Plana M, Ferrer E, Martinez C, et al. Immune restoration in HIV-positive, antiretroviral-naive patients after 1 year of zidovudine/lamivudine plus nelfinavir or nevirapine. *Antivir Ther.* 2004;2:197-204.
9. Malone JL, Simms TE, Gray GC, Wagner KF, Burge JR, Burke DS. Sources of variability in repeated T-helper lymphocyte counts from human immunodeficiency virus type 1-infected patients: total lymphocyte count fluctuations and diurnal cycle are important. *J Acq Immun Def Synd.* 1991;3:144-151.
10. Koepke J, Landay A. Precision and accuracy of absolute lymphocyte counts. *Clin Immunol Immunopathol.* 1989;52:19-27.

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