BD FACSPresto™ Cartridge

100 Tests—Catalog No. 657681

2/2016 23-12814-01

IVD

CE

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA

EC REP **Benex Limited**
Pottery Road, Dun Laoghaire,
Co. Dublin, Ireland
Tel +353.1.202.5222
Fax +353.1.202.5388

BD Biosciences
European Customer Support
Tel +32.2.400.98.95
Fax +32.2.401.70.94
help.biosciences@europe.bd.com

Becton Dickinson Pty Ltd,
4 Research Park Drive,
Macquarie University Research Park,
North Ryde NSW 2113, Australia

Becton Dickinson Limited,
8 Pacific Rise, Mt. Wellington,
Auckland, New Zealand

bdbiosciences.com
ClinicalApplications@bd.com

1. INTENDED USE

For use only with the BD FACSPresto™ Near-Patient CD4 Counter.

The BD FACSPresto Near-Patient CD4 Counter is an automated system for in vitro diagnostic use in performing the direct enumeration of CD4 absolute count, CD4 percentage of lymphocytes, and hemoglobin concentration in human whole blood.

Clinical Applications

CD4 counts and CD4 percentages (%CD4) have been used to evaluate the immune status of patients diagnosed with, or suspected of developing, immune deficiencies such as acquired immune deficiency syndrome (AIDS).[1,2]

The CD4 antigen is the receptor for the human immunodeficiency virus (HIV).[3] The absolute number and percentage of CD4 T lymphocytes are the cellular parameters most closely associated with HIV disease progression and patient prognosis.[4] The number of CD4 T lymphocytes declines in HIV infection.[5-7]

Hemoglobin is a protein in red blood cells that carries oxygen from the lungs to the body. Low or declining hemoglobin concentration is an indicator of anemia, a hematological abnormality frequently associated with HIV.[8-10]

2. PRINCIPLES OF THE PROCEDURE

The BD FACSPresto™ cartridge*, the CD4/%CD4/Hb cartridge, contains dried fluorochrome-conjugated antibody reagents. When blood reacts with the reagents, the antibodies in the reagents bind to the surface antigens on the

* BD FACSPresto Cartridge: US Patent 8,248,597

lymphocytes and monocytes. After the incubation period, the cells are analyzed on the BD FACSPresto Near-Patient CD4 Counter (the instrument). The software identifies the cell populations of interest and calculates CD4 absolute counts, CD4 percentages of lymphocytes, and hemoglobin concentration. The system measures total hemoglobin by a spectrophotometric method, using absorbance at an isobestic point for oxy-hemoglobin and deoxy-hemoglobin, with correction for scatter.

3. REAGENT COMPOSITION

The cartridge contains CD4 PE-Cy™5†/ CD3 APC/CD45RA APC/CD14 PE dried antibody reagents. The dried antibody reagents include inert ingredients such as buffer, bovine serum albumin (BSA), and ProClin®‡ as a preservative. CD3 APC and CD45RA APC enumerate total lymphocytes. Lymphocytes labeled with CD4 PE-Cy5 are designated $CD4^+$ lymphocytes. CD14 PE identifies monocytes, which are excluded from the analysis.

The CD4 antigen[11,12] (55 kDa)[13] is present on the helper/inducer T-lymphocyte subset[14,15] $CD3^+CD4^+$, which consists of 28% to 58%[16] of lymphocytes in normal peripheral blood,[12] and is present in low density on the surface and in the cytoplasm of monocytes. The CD4 antibody, clone SK3,[11] is derived from the hybridization of mouse myeloma cells with spleen cells from BALB/c mice immunized with human peripheral blood T lymphocytes. The CD4 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

The CD3 antibody, clone SK7, is derived from the hybridization of NS-1 mouse myeloma cells with spleen cells from BALB/c mice immunized with human thymocytes. CD3 is composed of mouse IgG_1 heavy chains and kappa light chains.

The CD45RA antigen is present on approximately 50% of $CD4^+$ T lymphocytes, approximately 75% of $CD8^+$ T lymphocytes, and on essentially all B lymphocytes and natural killer (NK) lymphocytes.[17] The helper/inducer T-lymphocyte subset expresses the phenotype $CD4^+CD45RA^+$.[17] The CD45RA antigen is expressed on naive T lymphocytes. Antigen density decreases upon in vitro activation.[18] A selective loss of the $CD4^+CD45RA^+$ subset during active multiple sclerosis has been demonstrated.[17,19]

The CD45RA antibody, clone HI100,[20] is derived from the hybridization of mouse myeloma cells with spleen cells isolated from mice immunized with human whole blood cells (WBCs).

The CD14 antigen is present on the majority of normal peripheral blood monocytes.[21] The CD14 antibody recognizes a human monocyte/ macrophage antigen of 55 kDa.[22] The CD14 antibody, clone MϕP9, is derived from the hybridization of Sp2/0 mouse myeloma cells with spleen cells from BALB/c mice immunized with peripheral

† Cy™ is a trademark of GE Healthcare. This product is subject to proprietary rights of GE Healthcare and Carnegie Mellon University, and is made and sold under license from GE Healthcare. This product is licensed for sale only for in vitro diagnostics. It is not licensed for any other use. If you require any additional license to use this product and do not have one, return this material, unopened, to BD Biosciences, 2350 Qume Drive, San Jose, CA 95131, and any money paid for the material will be refunded.

‡ ProClin is a registered trademark of Rohm and Haas Company.

blood monocytes from a patient with rheumatoid arthritis. The CD14 antibody is composed of mouse IgG_{2b} heavy chains and kappa light chains.

4. PACKAGE CONTENTS

Each box contains 100 cartridges and 100 pipets.

5. STORAGE AND HANDLING

Store the cartridge:

- In its original foil pouch. Do not use the cartridge if the pouch has been opened for more than 30 minutes.
- At 4°C–31°C (39°F–88°F).
- In 10%–95% non-condensing humidity.
- Until the expiration date. Do not use the cartridge after the expiration date on the package.

Incubate the cartridge at 10°C–40°C (50°F–104°F).

6. MATERIALS REQUIRED BUT NOT PROVIDED

- BD FACSPresto™ instrument
- BD FACSPresto™ work station
- BD FACSPresto™ finger stick sample collection kit
- BD Vacutainer® EDTA blood collection tubes

7. CARTRIDGE QC

Cartridge Quality Control (QC) uses immobilized antibodies. The instrument verifies that the reagent is present and that there is sufficient sample in the cartridge. Cartridge QC runs automatically at every cartridge run.

8. PATIENT SPECIMENS

The assay is designed to be used only with peripheral whole blood collected by venipuncture into EDTA tubes or by finger stick.

Capillary[23] or venous blood samples are transferred directly into the cartridge and incubated. Samples are run on the instrument after incubation.

Follow these guidelines for handling your samples:

- Do not dilute whole blood before adding it to the cartridge.
- Do not refrigerate the whole blood specimen before sample preparation.
- Store whole blood collected in EDTA tubes at 20°C–25°C (68°F–77°F) up to 24 hours before applying the blood to the cartridge.
- Minimize exposure of the cartridges to light.
- Do not remove the channel protector on the cartridge until just before you insert the cartridge into the instrument.

WARNING Do not use previously fixed and stored samples. Whole blood specimens refrigerated before staining can give incorrect results. Specimens from patients taking immunosuppressive drugs can yield poor resolution.[24] Do not test hemolyzed specimens.

WARNING The reagents contain antibodies of mouse and rat origin.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[25,26] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth.

Wear suitable protective clothing, eyewear, and gloves.

WARNING Each cartridge is for single use only. Use one cartridge per specimen.

9. PROCESS CONTROLS

For information about process controls or external quality assessment products, contact your local BD Biosciences office or representative.

10. PROCEDURE

See the following for instructions on preparing and running samples.

- *BD FACSPresto Near-Patient CD4 Counter Instructions for Use* (IFU)
- *BD FACSPresto Near-Patient CD4 Counter Quick Reference Guide*

11. REFERENCE INTERVALS

The reference intervals for the BD FACSPresto cartridge shown in Table 1 are representative for hematologically normal adults.

Table 1 Representative reference intervals

Subset	Gender	N	Mean	Reference interval
CD4[27]	Male	77	811	462–1,306 cells/µL
	Female	83	866	440–1,602 cells/µL
%CD4[27]	Male	77	41	29%–54%
	Female	83	44	32%–55%
Hb[28]	Male	NA	NA	13.5–18.0 g/dL
	Female	NA	NA	12.0–16.0 g/dL

We recommend that laboratories and other users establish their own reference intervals for their patient populations using the BD FACSPresto system to reflect potential sources of variability, such as patient gender, race, age, and preparation techniques.

12. EXPECTED RESULTS

Performance of the BD FACSPresto cartridge (the cartridge) was established by the testing at the BD Biosciences laboratories in San Jose, CA, USA and at one clinical laboratory in Kisumu, Kenya, Africa.

Method Comparison

Absolute counts of CD4-positive cells, the percentage of CD4 positive cells in the lymphocyte population, and total hemoglobin concentration in whole blood from HIV-infected patients were determined using the BD FACSPresto system. Results were compared with results from the BD Tritest™ CD3 FITC/CD4 PE/CD45 PerCP reagent, in BD Trucount™ tubes, on the BD FACSCalibur™ flow cytometer, with the BD FACS™ Loader, using BD Multiset™ software and the Sysmex®§ KX-21 hematology analyzer. Whole blood samples were collected at the clinical laboratories. Regression statistics reported in Table 2 and Table 3 indicate the results are substantially equivalent. Details are shown in Figure 1 through Figure 5.

Table 2 Method comparison for venous blood

Parameter	N	R^2	Slope	Intercept	Range
CD4	189	0.98	0.97	7.37	55–2,478 cells/µL
%CD4	188	0.96	1.03	0.13	5.06%–53.77%
Hb	190	0.96	0.94	0.18	3–18.9 g/dL

§ Sysmex is a registered trademark of Sysmex America Inc.

Table 3 Method comparison for capillary blood

Parameter	N	R^2	Slope	Intercept	Range
CD4	162	0.97	1.03	13.47	69–2,474 cells/µL
%CD4	161	0.96	1.02	-0.26	5.9%–56.2%

Regression analysis is not applicable for hemoglobin capillary samples. A total of 163 samples were collected with a range of 4.7–17.1 g/dL. The average bias around the medical decision level (9.5 g/dL–11.5 g/dL) for capillary hemoglobin specimens was calculated against Sysmex as 2.02%.

Figure 1 Scatter plot with weighted Deming fit (y = 0.97x + 7.37) for venous blood (CD4)

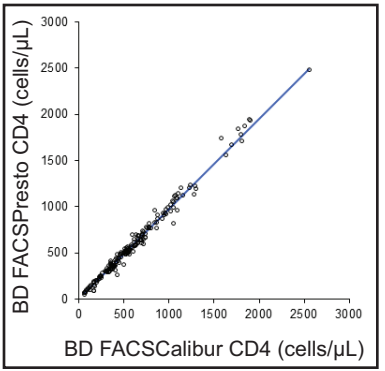


Figure 2 Scatter plot with Deming fit (y = 1.03x + 0.13) for venous blood (%CD4)

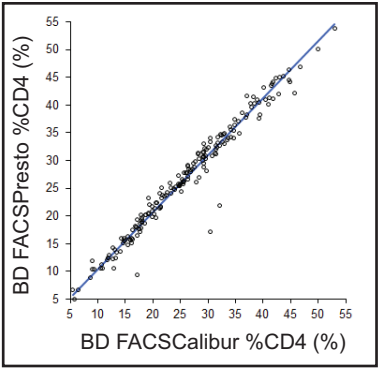


Figure 3 Scatter plot with weighted Deming fit (y = 1.03x + 13.47) for capillary blood (CD4)

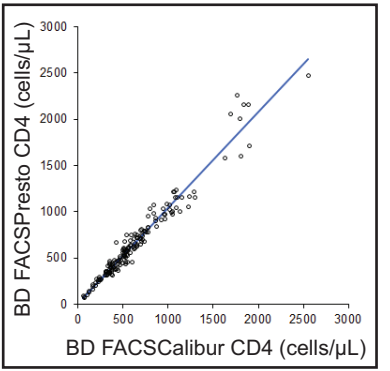


Figure 4 Scatter plot with Deming fit (y = 1.02x - 0.26) for capillary blood (%CD4)

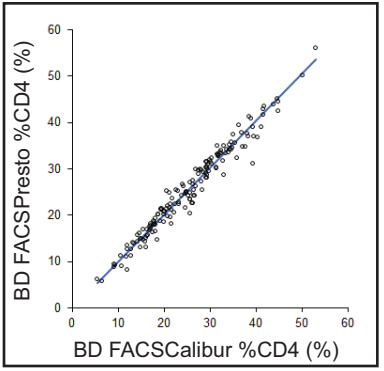
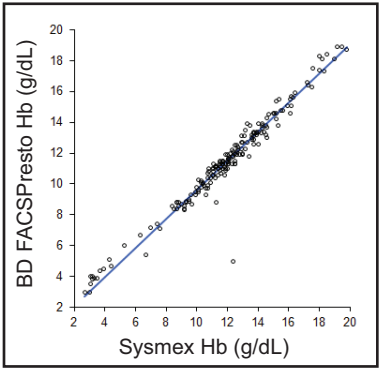


Figure 5 Scatter plot with Deming fit (y = 0.94x + 0.18) for venous blood (Hb)



Within-Specimen Reproducibility and Precision

Twenty replicates were created by one operator in one day, using two concentration levels of process controls, one lot of cartridges, and one instrument. Results are shown in Table 4.

Estimates of precision were determined at one site, BD Biosciences, using CD4 cellular and hemoglobin process controls. Two replicates of each CD4 control (normal and low) and two replicates of each of the 3 levels of hemoglobin controls were analyzed in each run, and two runs were performed per day for a total of 21 days. Three different instruments and three cartridge lots with three different operators were used, each for seven of the 21 days. Coefficients of variation (CVs) and standard deviations (SDs) are provided for CD4 absolute counts and CD4 percentages for within-run precision and total precision in Table 5 through Table 7, respectively.

Table 4 Repeatability study

	Level	Mean	CV	SD[a]	N
CD4	Low	155.10	5.78		20
	Normal	926.60	2.59		20
%CD4	Low	12.77		0.73	20
	Normal	43.99	1.53		20
Hb	Low	6.95	2.26		20
	Normal	12.99	1.09		20

a. Standard deviations (SDs) are reported instead of coefficients of variation (CVs) for %CD4 low level.

Table 5 Within-run and within-device precision CD4 absolute counts

	Low control (CV)	Normal control (CV)
Within-run	6.79	2.18
Within-device	6.79	3.30

Table 6 Within-run and within-device precision CD4 percentages

	Low control (SD[a])	Normal control (CV)
Within-run	0.75	1.58
Within-device	0.75	1.74

a. Standard deviations (SDs) are reported instead of coefficients of variation (CVs) for %CD4 low level.

Table 7 Within-run and within-device precision hemoglobin

	Low control (CV)	Medium control (CV)	High control (CV)
Within-run	2.42	1.46	1.07
Within-device	2.42	1.52	1.14

Stability

A stability study was conducted at the clinical laboratory in Kisumu to assess the following:

- Changes associated with the storage of whole blood before addition into the cartridge
- Changes as a result of time between addition of blood into the cartridge and data acquisition
- The combined effect of both

Whole blood samples were tested up to 24 hours post draw, and samples were tested up to 2 hours post addition of blood into the cartridge. All samples were maintained at room temperature (20°C–25°C, 68°F–77°F) before addition of blood into the cartridge or acquisition. Based on the results of this study, cartridges should be prepared with whole blood samples within 24 hours of draw, and then run within 2 hours of adding blood into the cartridge.

Linearity

Linearity was assessed in triplicate measurements of multiple concentrations of $CD4^+$ cells, lymphocyte cells, and hemoglobin across the reportable range of the assay for CD4 absolute counts, total lymphocytes, and hemoglobin on the instrument. Results are linear in the CD4 range (50–4,000 cells/µL), absolute lymphocyte range (200–10,000 cells/µL), and hemoglobin range (2–20 g/dL).

13. CARTRIDGE SPECIFICATIONS

Item	Description
Blood stability	Up to 24 hours after draw if stored in an EDTA tube at 20°C–25°C (68°F–77°F)
Sample stability	Up to 2 hours after addition of sample to cartridge
Sample throughput	More than 10 patient results per hour when run in batch mode
Validated range	CD4 count: 50–4,000 cells/µL %CD4: 5%–60% Hb concentration: 2.0–20 g/dL

14. LIMITATIONS

- Use the cartridge only with the BD FACSPresto instrument.
- The cartridge has no user-serviceable parts.
- Performance characteristics outside the validated range have not been established.
- Interfering substances in the sample may result in an inaccurate result.
- Follow the instructions in the IFU on preparing capillary and venous blood samples to ensure accurate results.

15. INTERFERING CONDITIONS

Table 8 lists the substances that were tested for interference with the reagents in the cartridge. Testing for interference was performed in accordance with EP7[29]. There was no detectable interference at the following concentrations.

Table 8 Interfering substances

Analyte	Max concentration
Acetaminophen	20 mg/dL
Albumin	6 g/dL
Amodiaquine	60 ng/mL
Artesunate	600 ng/mL
Ascorbic acid	6 mg/dL
Conjugated bilirubin	5 mg/dL
Creatinine	5 mg/dL
Efavirenz	16 µg/mL
Ethambutol	12 µg/mL
Gamma Globulin	40 mg/mL
Glucose	120 mg/dL
Hemolysis	20%
Ibuprofen	500 µg/mL
Iron	150 µg/dL
Isoniazid	40 µg/mL
Lipemia (intralipid)	2,400 mg/dL
Magnesium	6.3 µg/dL
Methemoglobin	14%
Nevirapine	7 µg/mL
Quinine	48 µg/mL
Rifampicin	64 µg/mL
Salicylic Acid	200 µg/mL
Tenofovir	1,000 ng/mL
Tetracycline	150 µg/mL
Thrombocytes (Platelets)	1.541 x 10^6 cells/µL

Table 8 Interfering substances

Analyte	Max concentration
Urea	40 mg/dL
Uric acid	9 mg/dL
White Blood Cells	25 x 10^3 cells/µL
Zidovudine	1,000 ng/mL
Disease Condition	
Rouleaux Formation	
Cold Agglutinin	

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

THE PRODUCTS SOLD HEREUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. BD'S SOLE LIABILITY IS LIMITED TO EITHER REPLACEMENT OF THE PRODUCTS OR REFUND OF THE PURCHASE PRICE. BD IS NOT LIABLE FOR PROPERTY DAMAGE OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.

TROUBLESHOOTING

See the troubleshooting section in the *BD FACSPresto Near-Patient CD4 Counter Instructions For Use*.

REFERENCES

1. Giorgi JV, Hultin LE. Lymphocyte subset alterations and immunophenotyping by flow cytometry in HIV disease. *Clinical Immunology Newsletter.* 1990;10:55-61.
2. Schmidt RE. Monoclonal antibodies for diagnosis of immunodeficiencies. *Blut.* 1989;59:200-206.
3. Dalgleish AG, Beverley PC, Clapham PR, Crawford DH, Greaves MF, Weiss RA. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature.* 1984;312:763-767.
4. Fahey JL, Taylor JM, Detels R, et al. The prognostic value of cellular and serologic markers in infection

with human immunodeficiency virus type 1. *N Engl J Med*. 1990;322:166-172.

5. Lewis DE, Puck JM, Babcock GF, Rich RR. Disproportionate expansion of a minor T cell subset in patients with lymphadenopathy syndrome and acquired immunodeficiency syndrome. *J Infect Dis*. 1985;151:555-559.
6. Ohno T, Kanoh T, Suzuki T, et al. Comparative analysis of lymphocyte phenotypes between carriers of human immunodeficiency virus (HIV) and adult patients with primary immunodeficiency using two-color immunofluorescence flow cytometry. *Tohoku J Exp Med*. 1988;154:157-172.
7. Stites DP, Casavant CH, McHugh TM, et al. Flow cytometric analysis of lymphocyte phenotypes in AIDS using monoclonal antibodies and simultaneous dual immunofluorescence. *Clin Immunol Immunopathol*. 1986;38:161-177.
8. Henry DH, Beall GN, Benson CA, et al. Recombinant human erythropoietin in the treatment of anemia associated with human immunodeficiency virus (HIV) infection and zidovudine therapy. Overview of four clinical trials. *Ann Intern Med*. 1992;117:739-748.
9. Firnhaber C, Smeaton L, Saukila N, et al. Comparisons of anemia, thrombocytopenia, and neutropenia at initiation of HIV antiretroviral therapy in Africa, Asia, and the Americas. *Int J Infect Dis*. 2010; 14:e1088-1092.
10. Levine AM, Berhane K, Masri-Lavine L, et al. Prevalence and correlates of anemia in a large cohort of HIV-infected women: Women's Interagency HIV Study. *J Acquir Immune Defic Syndr.* 2001,26:28-35.
11. Bernard A, Boumsell L, Hill C. Joint report of the first international workshop on human leucocyte differentiation antigens by the investigators of the participating laboratories. In: Bernard A, Boumsell L, Dausset J, Milstein C, Schlossman SF, eds. *Leucocyte Typing*. New York, NY: Springer-Verlag; 1984:9-108.
12. Evans RL, Wall DW, Platsoucas CD, et al. Thymus-dependent membrane antigens in man: inhibition of cell-mediated lympholysis by monoclonal antibodies to TH2 antigen. *Proc Natl Acad Sci USA*. 1981;78:544-548.
13. Ledbetter JA, Evans RL, Lipinski M, Cunningham-Rundles C, Good RA, Herzenberg LA. Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. *J Exp Med*. 1981;153:310-323.
14. Engleman EG, Benike CJ, Glickman E, Evans RL. Antibodies to membrane structures that distinguish suppressor/cytotoxic and helper T lymphocyte subpopulations block the mixed leukocyte reaction in man. *J Exp Med*. 1981;154:193-198.
15. Kotzin BL, Benike CJ, Engleman EG. Induction of immunoglobulin-secreting cells in the allogeneic mixed leukocyte reaction: regulation by helper and suppressor lymphocyte subsets in man. *J Immunol*. 1981;127:931-935.
16. Reichert T, DeBruyère M, Deneys V, et al. Lymphocyte subset reference ranges in adult Caucasians. *Clin Immunol Immunopath*. 1991;60:190-208.
17. Sobel RA, Hafler DA, Castro EA, Morimoto C, Weiner HL. The 2H4 (CD45R) antigen is selectively decreased in multiple sclerosis lesions. *J Immunol*. 1988;140:2210-2214.
18. Serra HM, Krowka JF, Ledbetter JA, Pilarski LM. Loss of CD45R (Lp220) represents a post-thymic T cell differentiation event. *J Immunol*. 1988;140:1435-1441.
19. Rose LM, Ginsberg AH, Rothstein TL, Ledbetter JA, Clark EA. Selective loss of a subset of T helper cells in active multiple sclerosis. *Proc Natl Acad Sci USA*. 1985;82:7389-7393.
20. Schwinzer R. Cluster report: CD45/CD45R. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1989:628-634.
21. Bernstein ID, Self S. Joint report of the myeloid section of the Second International Workshop on Human Leukocyte Differentiation Antigens. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human Myeloid and Hematopoietic Cells*. Vol 3. New York, NY: Springer-Verlag; 1986:1-25.
22. Goyert SM, Ferrero E. Biochemical analysis of myeloid antigens and cDNA expression of gp55 (CD14). In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1987:613-619.
23. *Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Sixth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2008. CLSI document GP42-A6.
24. Giorgi JV. Lymphocyte subset measurements: significance in clinical medicine. In: Rose NR, Friedman H, Fahey JL, eds. *Manual of Clinical Laboratory Immunology*. 3rd ed. Washington, DC: American Society for Microbiology; 1986:236-246.
25. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Third Edition*. Wayne, PA: Clinical and

Laboratory Standards Institute; 2005. CLSI document M29-A3.

26. Siegel JD, Rhinehart E, Jackson M, Chiarello L, Healthcare Infection Control Practices Advisory Committee. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. http://www.cdc.gov/ncidod/dhqp/pdf/isolation2007.pdf.
27. Zeh C, Amornkul PN, Inzaule S, et al. Population-based biochemistry, immunologic and hematological reference values for adolescents and young adults in a rural population in western Kenya. *PLOS One*. 2011;6:1-10.
28. Lehmann HP, Henry JB. SI Units. In: Henry, J, ed. *Clinical Diagnosis and Management Diagnosis, Twentieth Edition*. Philadelphia, PA: W.B. Saunders Company; 2001: 1426-1441.
29. McEnroe RJ, Burritt, MF, Powers DM, et al. *Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI document EP7-A2.