IRBAS www.irbas.academyirmbr.com January 2022
International Review of Basic and Applied Sciences Vol. 10 Issue.1

Evaluation of Single and Dual Platforms for CD4 and CD8 T-Lymphocyte Count among HIV Patients

HAYTHAM DAHLAWI
Clinical Laboratory Sciences Department, College of Applied Medical Sciences,
Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia.
Email: haythamdahlawi@gmail.com

Abstract

HIV is a major problem worldwide affecting thousands of people each year. CD4 T cells and CD8 T cells counts are important factors for assessment immune system and monitoring and management immune status of HIV-infected people. The main methodologies available for absolute CD4 T cells and CD8 T cells analysis are the dual-platform and single-platform. Studies demonstrated grater performance by single-platform method laboratories on percentage of T-cell subset percentage for accuracy and measurements for precision However, some dual-platform method laboratories outperformed all single-platform method laboratories on percentages of T-cell subset in other studies. Therefore, the goal of this study is to identify whether or not it is necessary to change from dual-platform to single-platform techniques by determine the variation of relative counts for CD4 T cells and CD8 T cells Data set consists of 85 HIV patients have been studies. Two methods have been compared to indicate CD4 T cells to CD8 T cells counts, dual-platform method using hematology analyzer to estimate absolute lymphocyte count and the percentage of CD4 T cells among lymphocytes obtained from flow cytometry and single-platform method enables absolute CD4 T cell counts to be measured by flow cytometry directly. This study showed statistically significant differences between the single-platform and dual-platform technology in favor of single-platform. The percentage of differences in CD4 and CD8 values between methods were 8% and 10% and somehow similar trend was noticed for each of CD4 single- and dual-platform as well as CD8 single- and dual-platform when it comes to parallel coordinates comparisons. Our study suggesting a major disadvantage of dual-platform method might be error in each independent measurement affecting each following step in the T- cell calculation values. While high precision in single-platform method is due to depending only on performance of one measurement. However, further investigations and studies are required to compare the accuracy and precision of both techniques.

Keywords: *Patients, HIV, CD4 T Cells, CD8 T Cells, Dual-platform, Single-platform.*

Introduction

Human immunodeficiency virus (HIV) is considered the etiologic agent of acquired immunodeficiency syndrome (AIDS), a disease that has posed one of the major problem worldwide and greatest medical challenges affecting health development and reducing life expectancy (Woodham et al., 2016)(Maina et al., 2020). Aalthough the most of infected people live in developing countries, HIV infection also has caused a significant medical problems in developed nations (Woodham et al., 2016). Since, the first cases of HIV were reported in 1981, infection has developed to pandemic proportions, causing more than 79.3 million and 36.3 deaths (WHO, 2020). According to the World Health Organization (WHO), an estimated 1.5 million people became newly infected with HIV. Of the estimated 37 million people were living with HIV worldwide, nearly 53% are women and girls in 2020 (WHO, 2020). Thus, precise diagnosis is critical for

early intervention and halting the spread of HIV infection, and the need for affordable diagnostic and monitoring tools for the treatment of HIV still a priority goal.

Once a treatment of patients with HIV infection has been started, it is important to monitor patients over time to assess the efficacy of antiretroviral therapy (ART) (Althoff et al., 2016). This way, signs of disease development can be identified early and assisting to decisions about further treatment strategy. CD4 T cells and CD8 T cells counts are essential elements for immune system for monitoring and management immune status in infected patients with HIV (Milanés-Guisado et al., 2018)(Li et al., 2009)(Serrano-Villar et al., 2015).

CD4 T cells destruction is vital to the immunopathogenesis of HIV infection and a significant decrease in CD4 T cells has long been known as the hallmark characteristic of AIDS. Thus, number of CD4 T cells in the peripheral blood has showed a fundamental role in assessing prognosis and the level of immune suppression to determine the start of ART in patients infected with HIV (Rice et al., 2019).

In addition to CD4 T cells absolute counts and percentages, CD4 T cells to CD8 T cells ratio might be reported to evaluate the dynamics between the two T-cell lineages. In patients infected with HIV, mostly those with AIDS, the significant decrease in the of CD4 T cells count, together with an increase in CD8 T cells, caused an inverted ratio (Lu et al., 2015).

CD4 T cells counts can vary from day to day and non-pathological differences have been detected in measurement of these cells , therefore technical variations in methodologies must be decreased to minimize the amount of possible variables. Variations in normal ranges, and how these may influence a doctor's decision about treatment of HIV patients, is a concern. Frequently, patients in severe stages of HIV have significant decrease in CD4 T cells counts, and a small change may demonstrate significant in the close monitoring of disease development (Connelly et al., 1995).

Currently, immunophenotyping analysis by flow cytometry is the gold standard for CD4 T cells counts. The main methodologies available for absolute CD4 T cells and CD8 T cells analysis are the dual-platform and single-platform (World Health Organization, 2010)(Nasi et al., 2015).

Dual-platform technology involves two separate parameters; hematology analyser to estimate absolute lymphocyte count and the percentage of CD4 T cells among lymphocytes obtained from flow cytometry. The single-platform method enables absolute CD4 T cell counts to be measured directly with a single parameter (flow cytometry) (Hultin et al., 2010)(Bainbridge et al., 2014).

Studies suggest single-platform approach as the favoured technology for absolute counting to minimize inter-laboratory coefficients of variation. Actually, researchers reported a noticeable progression in CD4 T cells absolute counts between laboratories, with lesser interlaboratory variations, when comparing single-platform and dual-platform approaches (Hultin et al., 2010; Sepstrup & Sargent, 2003).

Also, Study has compared dual-platform method to single-platform method laboratories demonstrated grater performance by single-platform method laboratories on percentage of T-cell subset percentage for accuracy and measurements for precision (Whitby et al., 2013). However, in dual-platform method results high degree of interlaboratory variability was observed, and some dual-platform method laboratories outperformed all single-platform method laboratories on percentages of T-cell subset (Bainbridge et al., 2014).

Thus, the goal of this study is to identify whether or not it is necessary to change from dual-platform to single-platform techniques by determine the variation of relative counts for CD4 T cells and CD8 T cells.

Material and Methods

Specimen Collection and preparation

Whole blood specimens was collected aseptically by venepuncture from 85 HIV patients at western region of Saudi Arabia, using BD Vacutainer™ blood collection tubes. Anticoagulated blood were stored at room temperature (20° to 25°C) and stained within less than 12 hours of draw and analysed within 1 hours of staining. Staining the Cells Lyse red blood cells following staining using diluted (1X) BD FACS lysing solution. Procedure was performed at room temperature (20° to 25°C). For each patient sample, Trucount tube was labelled with the sample identification number. Then 20 µL of BD Multitest 6-color TBNK reagent added into the bottom of the tube. After that, 50 µL of well mixed, anticoagulated whole blood were added into the bottom of the tube (reverse pipetting technique was used to add accurate volume to Trucount tubes), then mixed gently using vortex. Following mixing, samples incubated for 15 minutes in the dark at room temperature (20° to 25°C). Then 450 µL of 1X BD FACS lysing solution was added to the tube, mixed gently, incubated for 15 minutes in the dark at room temperature (20° to 25°C) and analysed within 1 hours.

Quality Control

In accordance with the College of American Pathologists (CAP) guidelines, two levels of liquid control materials (procedural control) were processed like patient samples to monitor the outgoing performance of the entire analytic process. This was done once each day patient testing was performed.

Analysis

CD45 vs SSC dot plot was visually inspected. Lymphocyte population should appear as a bright, compact cluster with low SSC. Monocytes and granulocytes also should appear as distinct clusters. Analysis was not proceeded if populations were diffused and if there was little or no separation between clusters.

Calculating Absolute Counts using Single platform technique: Using BD FACSCanto clinical software, results were appeared as a percentage of lymphocytes and as absolute count (per microliter of blood). Calculating Absolute Counts using dual platform technique: The dual-platform counting method multiplies the flow cytometry-derived lymph subset cell percentage by the absolute lymphocyte count derived from the hematology analyser to calculate the absolute count of lymphocytes subset (Hematology Analyzer used was Abbott Alinity hq, Abbott, USA).

Statistical Analysis

As a preliminary step to get insight into the data set, the Shapiro-Wilk test of normality was performed (Royston, 1995) to the four variables (CD4 Single, CD4 Dual, CD8 Single, CD8 Dual). This normality test proved that all the four variables. Therefore, Wilcoxon tests (non-parametric version of t-test) was carried out (Hollander & Wolfe, 1999). All statistical analyses were carried using R Language & Environment for Statistical Computing R Core Team (2021).

Results

This data set consists of 85 HIV patients, their ages averaged 49.5 years, where ranged from 9 to 69 years (Figure 1). It is also worth mentioning, if we ignored the 9 years old patient (an outlier) from the data set, the age will range from 30 to 69. There were 29 females and 56 males in the data set (Figure 2).

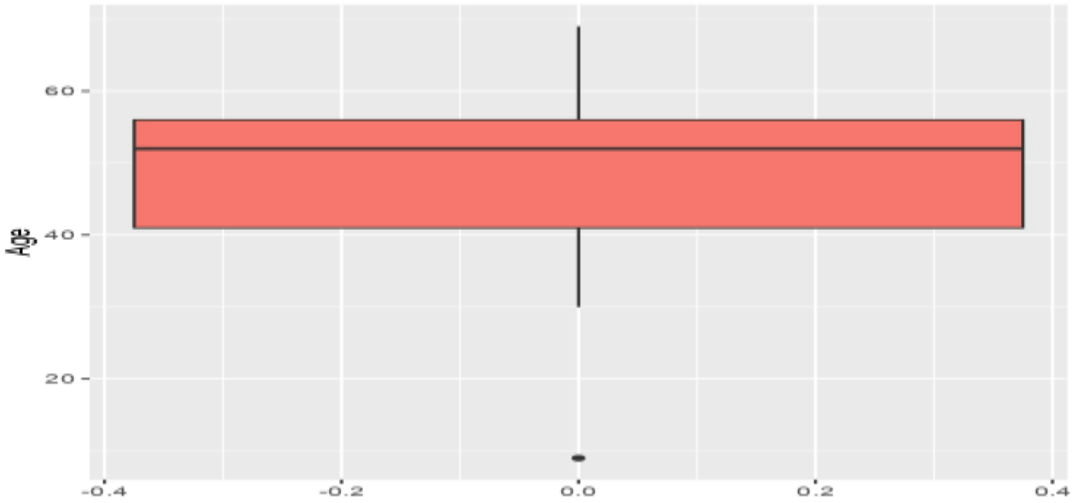


Figure 1: Boxplot of ages, top and bottom of each vertical lines represent the maximum and minimum ages, the lower and upper bounds of each box represent the first quartile *(25th* percentile) and third quartile *(75th* percentile) of the ages where the line inside box is the median *(2ed* quartile Q2), the dot in the very bottom of the figure is an outlier.

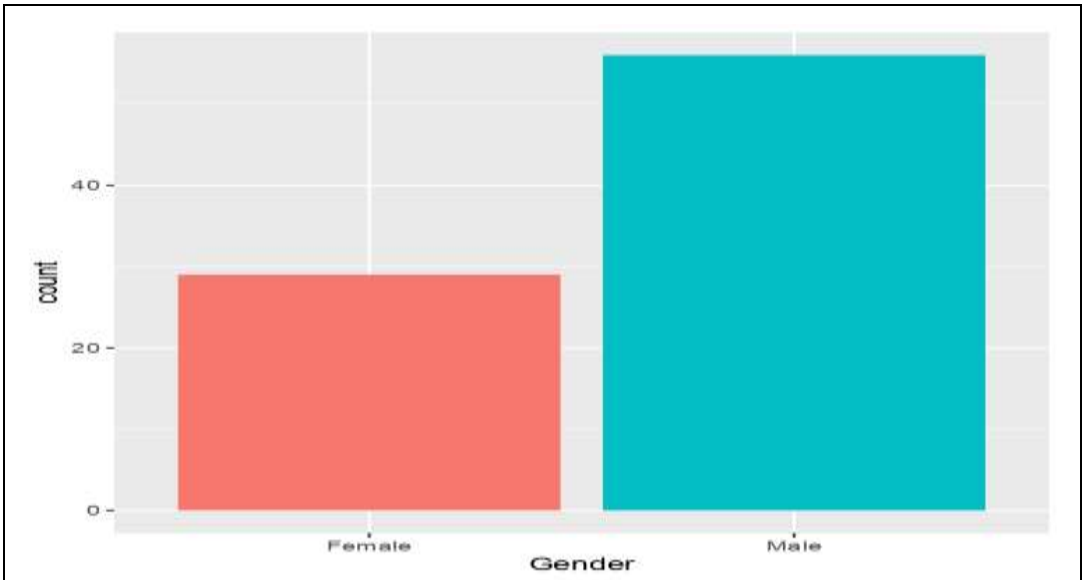


Figure 2: Number of females and males in the data set

Boxplot of CD4 Single, CD4 Dual, CD8 Single, and CD8 Dual is presented in Figure (3), where top and bottom each vertical lines represent the maximum and minimum values, the top and bottom of each box represent the first quartile (Ql) and third quartile (Q3) where the line inside each box notch is the median (second quartile Q2), dots around the vertical lines represent the data points. No outliers were noticed for CD4-dual methodology.

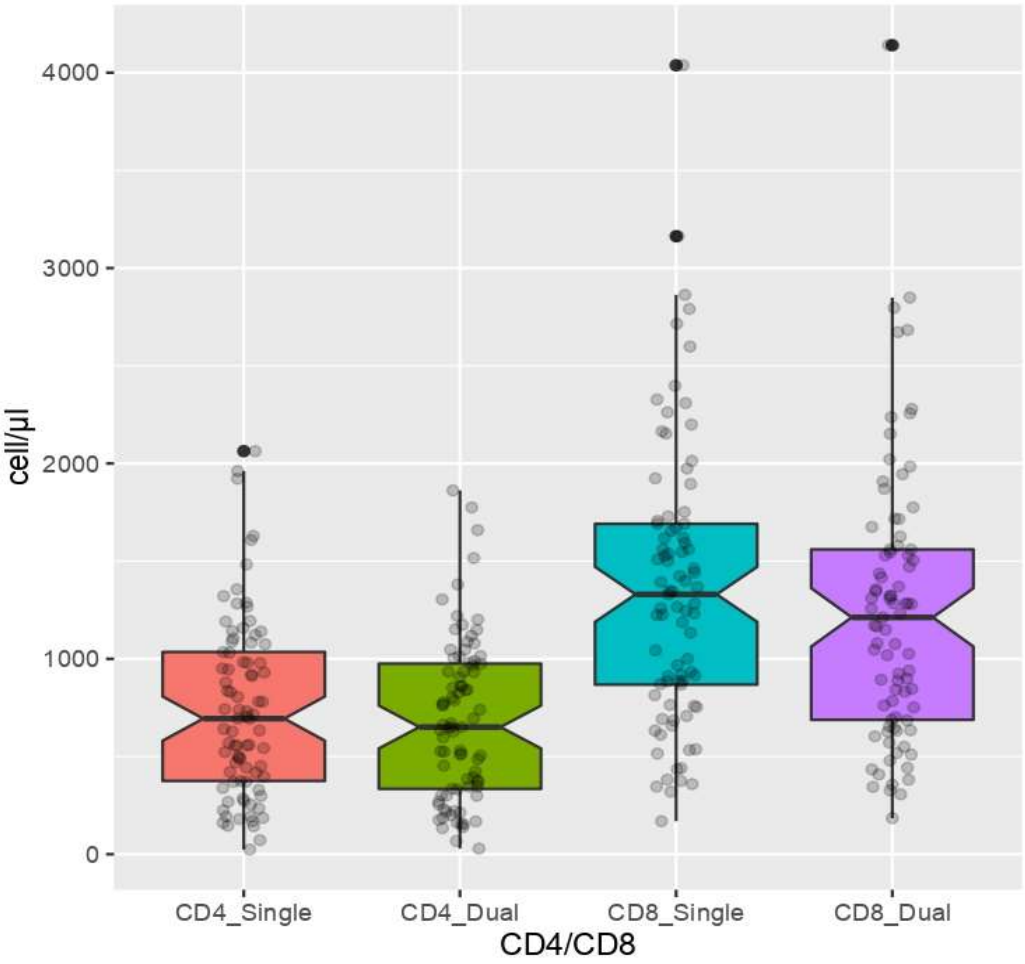
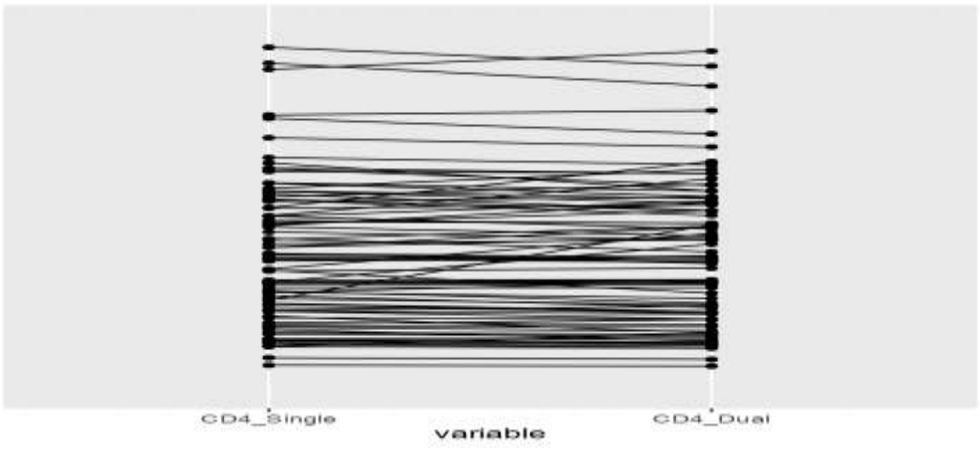


Figure 3: Boxplot of CD4 Single, CD4 Dual, CD8 Single, and CD8 Dual, top and bottom of each vertical lines represent the maximum and minimum values, the top and bottom of each box represent the first quartile (Ql) and third quartile (Q3) where the line inside each box notch is the median (second quartile Q2), dots around the vertical lines represent the data points.

Means, standard deviations, ranges, non-parametric *t* values, and P values for CD4 and CD8 single- and dual-platform methodologies are listed in table (1). All moments (e.g., mean, SD and median) were higher in favor of single methodology. There were statistically significant differences between the single-platform and dual-platform technology in favor of single-platform. The percentage of differences in CD4 and CD8 values between methods were 8% and 10%.

Table No 1: Descriptive Statistics

	Mean	SD	Median	Range	t-value	P value
CD4 single	741.3	454.6	694	24-2063	3084	<0.0001
CD4 dual	684.6	412.5	651	29-1862		
CD8 single	1357.6	719.3	1330	169-4038	3246	<0.0001
CD8 dual	1239.7	695.5	1239.7	185-4141		



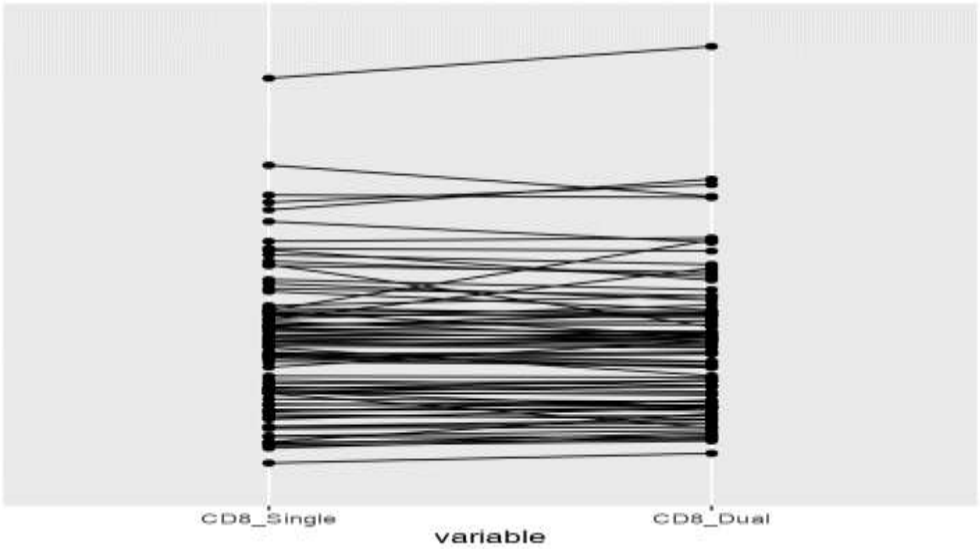


Figure 4: Individual comparison for CD4- and CD8 single and dual platforms; (a) Individual comparisons of CD4 single versus dual data (b) Individual comparisons of CD8 single versus dual data.

The values obtained for single- and dual-platform methodologies on each individual test are shown for CD4 (4a) and (4b) in a form of parallel coordinates comparisons. In general, the highest values for each of CD4 and CD8 single platform exhibited larger discrepancy than the Mean SD and median. In general a somehow similar trend was noticed for each of CD4 single- and dual-platform as well as CD8 single- and dual-platform when it comes to parallel coordinates comparisons.

Discussion

HIV is a major problem worldwide affecting thousand of people each year. CD4 T cells and CD8 T cells counts are important factors for assessment immune system and monitoring and management immune status of HIV-infected people. Despite individual differences in CD4 T cells and CD8 T cells counts in this study were small when comparing the two methods; dual-platform and single platform, there were minor

but statistically significant differences between the single-platform and dual-platform technology in favor of single-platform and somehow similar trend was noticed for each of CD4 single- and dual-platform as well as CD8 single- and dual-platform when it comes to parallel coordinates comparisons. Bainbridge et al (2014) reported high performance by single platform method laboratories on T-cell subset percentages for accuracy and measurements for precision (Bainbridge et al., 2014). Similarly Sepstruo and Sargent, showed that data obtained with single platform method was significantly higher than dual-platform for both CD4 T cells and CD8 T cells, suggesting importance for clinicians to carefully monitor CD4 T cells and CD8 T cells counts and notified for any laboratory changes (Sepstrup & Sargent, 2003).

Another study of 18 HIV patients (one female, 17 male) demonstrated a good correlation between single-platform and dual-platform, however CD4 T cells counts were showed as higher using the dual-platform technology, suggesting doctors need to be aware of such differences when interpreting results from patients entering trials where the another method is used (Jeganathan et al., 2008). Also, Higgins et al (2007), reported good correlation for CD4 T cells and CD8 T cells values, preferring dual-platform over single-platform until single-platform become more practicable (Higgins et al., 2007). Study performed in Multicenter AIDS Cohort Study (MACS) laboratories found significant degree of interlaboratory variability in dual-platform technology results and dual-platform technology laboratory outperformed all single-platform method on percentages of T-cell subset.

Studies demonstrated that laboratory bias in CD4 T cells values rely on hematology analyzer used to generate absolute lymphocyte count. In addition, due to different hematology analysers using its own principle and technology in differentiating leukocytes and measuring absolute counts, there is a variation in WBC and absolute lymphocyte counts (Sepstrup & Sargent, 2003) . Moreover An important variance between two methodologies is that hematology analyzers use fixed samples volume, while predefined number of cell events are analysed by flow cytometry (Sepstrup & Sargent, 2003). Also long time between blood collection and measurement by dual-platform method laboratory's hematology analyzers has been associated with variance between single-platform method and dual-platform method for percentage measurements not for absolute count, as it has been shown that testing sample more than 6 hours after blood drawn falsely increase the count of lymphocyte because of degranulation of granulocytes (Bainbridge et al., 2014)(Calvelli et al., 1993).

In addition, gating strategy in flow cytometry for selecting lymphocyte increase the accuracy of CD4 T cells final counts. Study demonstrated that clinical laboratories that not using CD45 gating strategy have 2 to 3 times more possibility of giving inaccurate result of CD4 T cells values (Janossy et al., 2002). Moreover, monoclonal antibodies for selection lymphocytes that are not used by majority of hematology analysers, they can recognize leukocyte populations more accurate than forward and side scatter method (Janossy et al., 2002).

Although a general progression in assay precision of single platform methods, the performance of different laboratories varied considerably. Thus, a number of practical points need to be addressed. Reimann et al (2000), reported varied in performance of individual laboratories that participate when comparing CD4 T cells and CD8 T cells count by single versus dual-platform methods, as only 3 of the five participating laboratories, when assessed individually, demonstrated significant improvement in within-laboratory precision using single-platform (Reimann et al., 2000). Also, Precise pipetting is an important technique in single-platform technology than that need for dual- platform method as accurate results depend on precise 1:1 volume ration of blood and fluorosphere suspension, also positive displacement pipettes should always be used in this method (Reimann et al., 2000). Furthermore, Well mixing of beads that used for absolute counting must be done each time by technician. Thus, all technicians must perform the same technique when mixing beads before using them in single-platform methodology (Higgins et al., 2007).

Conclusion

Our study suggesting a major disadvantage of dual-platform method might be error in each independent measurement affecting each following step in the T- cell calculation values. While high precision in single-platform method is due to depending only on performance of one measurement. Clinical laboratories wishing to change from dual platform to single platform absolute counts should be concerned of a potential bias between the technologies and take the suitable measures to implement the switching to new technology. Also, it is essential that doctors closely monitoring T cells absolute count are informed of any laboratories changes. As any differences in T cells absolute values resulted by different techniques might cause an argument when used in diagnosis and treatment strategies of patients. However, further investigations and studies are required to compare the accuracy and precision of both techniques.

References

Althoff, K. N., Smit, M., Reiss, P., & Justice, A. C. (2016). HIV and ageing: Improving quantity and quality of life. In *Current Opinion in HIV and AIDS* (Vol. 11, Issue 5). https://doi.org/10.1097/COH.0000000000000305

Bainbridge, J., Wilkening, C. L., Rountree, W., Louzao, R., Wong, J., Perza, N., Garcia, A., & Denny, T. N. (2014). The Immunology Quality Assessment Proficiency Testing Program for CD3+4+ and CD3+8+ lymphocyte subsets: A ten year review via longitudinal mixed effects modeling. In *Journal of Immunological Methods* (Vol. 409). https://doi.org/10.1016/j.jim.2014.05.017

Calvelli, T., Denny, T. N., Paxton, H., Gelman, R., & Kagan, J. (1993). Guideline for flow cytometric immunophenotyping: A report from the national institute of allergy and infectious diseases, division of AIDS. *Cytometry*, *14*(7). https://doi.org/10.1002/cyto.990140703

Connelly, M. C., Knight, M., Giorgi, J. V, Kagan, J., Landay, A. L., Parker, J. W., Page, E., Spino, C., Wilkening, C., & Mercolino, T. J. (1995). Standardization of absolute CD4+ lymphocyte counts across laboratories: an evaluation of the Ortho CytoronAbsolute flow cytometry system on normal donors. *Cytometry: The Journal of the International Society for Analytical Cytology*, *22*(3), 200–210.

Higgins, J., Hill, V., Lau, K., Simpson, V., Roayaei, J., Klabansky, R., Stevens, R. A., Metcalf, J. A., & Baseler, M. (2007). Evaluation of a single-platform technology for lymphocyte immunophenotyping. *Clinical and Vaccine Immunology*, *14*(10). https://doi.org/10.1128/CVI.00168-07

Hultin, L. E., Chow, M., Jamieson, B. D., O'Gorman, M. R. G., Menendez, F. A., Borowski, L., Denny, T. N., & Margolick, J. B. (2010). Comparison of interlaboratory variation in absolute T-cell counts by single-platform and optimized dual-platform methods. *Cytometry Part B - Clinical Cytometry*, *78*(3). https://doi.org/10.1002/cyto.b.20500

Janossy, G., Jani, I. V., Bradley, N. J., Bikoue, A., Pitfield, T., & Glencross, D. K. (2002). Affordable CD4+-T-cell counting by flow cytometry: CD45 gating for volumetric analysis. *Clinical and Diagnostic Laboratory Immunology*, *9*(5). https://doi.org/10.1128/CDLI.9.5.1085-1094.2002

Jeganathan, S., Bansal, M., Smith, D. E., & Gold, J. (2008). Comparison of different methodologies for CD4 estimation in a clinical setting. *HIV Medicine*, *9*(4). https://doi.org/10.1111/j.1468-1293.2008.00544.x

Li, X., Breukers, C., Ymeti, A., Lunter, B., Terstappen, L. W. M. M., & Greve, J. (2009). CD4 and CD8 enumeration for HIV monitoring in resource-constrained settings. *Cytometry Part B - Clinical Cytometry*, *76*(2). https://doi.org/10.1002/cyto.b.20445

Lu, W., Mehraj, V., Vyboh, K., Cao, W., Li, T., & Routy, J. P. (2015). CD4:CD8 ratio as a frontier marker for clinical outcome, immune dysfunction and viral reservoir size in virologically suppressed HIV-positive patients. In *Journal of the International AIDS Society* (Vol. 18, Issue 1). https://doi.org/10.7448/IAS.18.1.20052

Maina, E. K., Adan, A. A., Mureithi, H., Muriuki, J., & Lwembe, R. M. (2020). A Review of Current Strategies Towards the Elimination of Latent HIV-1 and Subsequent HIV-1 Cure. *Current HIV Research*, *19*(1). https://doi.org/10.2174/1570162x18999200819172009

Milanés-Guisado, Y., Gutiérrez-Valencia, A., Trujillo-Rodríguez, M., Espinosa, N., Viciana, P., & López-

Cortés, L. F. (2018). Absolute CD4+ T cell count overstate immune recovery assessed by CD4+/CD8+ ratio in HIV-infected patients on treatment. *PLoS ONE*, *13*(10). https://doi.org/10.1371/journal.pone.0205777

Nasi, M., De Biasi, S., Bianchini, E., Gibellini, L., Pinti, M., Scacchetti, T., Trenti, T., Borghi, V., Mussini, C., & Cossarizza, A. (2015). Reliable and accurate CD4+ T cell count and percent by the portable flow cytometer cyflow minipoc and "CD4 easy count kit- Dry", as revealed by the comparison with the gold standard dual platform technology. *PLoS ONE*, *10*(1). https://doi.org/10.1371/journal.pone.0116848

Reimann, K. A., O'Gorman, M. R. G., Spritzler, J., Wilkening, C. L., Sabath, D. E., Helm, K., Campbell, D. E., O'Gorman, M., Bessent, E., Plaeger, S., Donnenberg, A., Douglas, S., Mandy, F., Nicholson, J., Reimann, K., Schmitz, J., Schnizlein-Bick, C., Kagan, J., & Livnat, D. (2000). Multisite comparison of CD4 and CD8 T-lymphocyte counting by single- versus multiple-platform methodologies: Evaluation of Beckman Coulter Flow- Count fluorospheres and the tetraONE System. *Clinical and Diagnostic Laboratory Immunology*, *7*(3). https://doi.org/10.1128/CDLI.7.3.344-351.2000

Rice, B., Boulle, A., Schwarcz, S., Shroufi, A., Rutherford, G., & Hargreaves, J. (2019). The continuing value of CD4 cell count monitoring for differential HIV care and surveillance. In *JMIR Public Health and Surveillance* (Vol. 5, Issue 1). https://doi.org/10.2196/11136

Sepstrup, S. E., & Sargent, J. M. (2003). Change from dual- to single-platform reporting of CD4/CD8 values: Experience from a small district general hospital laboratory. *British Journal of Biomedical Science*, *60*(2). https://doi.org/10.1080/09674845.2003.11783681

Serrano-Villar, S., Sainz, T., & Moreno, S. (2015). Monitoring the CD4/CD8 ratio: A promising indicator of disease progression in HIV-infected individuals? In *Future Virology* (Vol. 10, Issue 1). https://doi.org/10.2217/fvl.14.85

Whitby, L., Whitby, A., Fletcher, M., Helbert, M., Reilly, J. T., & Barnett, D. (2013). Comparison of methodological data measurement limits in CD4+ T lymphocyte flow cytometric enumeration and their clinical impact on HIV management. *Cytometry Part B - Clinical Cytometry*, *84*(4). https://doi.org/10.1002/cyto.b.21094

WHO. (2020). Global HIV & AIDS statistics. Fact sheet. In *Unaids*.

Woodham, A. W., Skeate, J. G., Sanna, A. M., Taylor, J. R., Da Silva, D. M., Cannon, P. M., & Martin Kast, W. (2016). Human Immunodeficiency Virus Immune Cell Receptors, Coreceptors, and Cofactors: Implications for Prevention and Treatment. *AIDS Patient Care and STDs*, *30*(7). https://doi.org/10.1089/apc.2016.0100

World Health Organization. (2010). Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach. 2010 Revision. In *Health (San Francisco)*.