DOI : 10.5281/zenodo.15318008 **SHORT COMMUNICATION**

WoSciMIB **01** (2025) 1-8

Optimizing whole blood stimulation conditions for intracellular cytokine staining: A solution for resource-limited laboratories and field studies

Gnodja Tégmaba[1,2*], Kadanga Motomdéwa Monique[1], Bara Fagdéba David[1], Simfele Hombamane Christelle[1], Kolou Maléwé[3], Mbengue Babacar[2]

[1]Unité de Recherche en Immunologie et Immunomodulation (UR2IM) /Laboratoire de Microbiologie et de Contrôle de Qualité des Denrées Alimentaires (LAMICODA)/Ecole Supérieure des Techniques Biologiques et Alimentaires (ESTBA), Université de Lomé, Lomé-Togo
[2]Service d'Immunologie, Faculté de Médecine, de Pharmacie et d'Odontologie (FMPOS), Université Cheikh Anta Diop (UCAD), Dakar-Sénégal
[3]Faculté des Sciences de la Santé (FSS), Université de Lomé, Lomé-Togo

Edited by

Tchadie Edlom Pelagie, University of Tübingen, Germany

***Corresponding author**

Gnodja Tégmaba
tegmabagno@gmail.com

Received: 11 February 2025

Accepted: 15 April 2025

Published: 01 May 2025

Citation

Gnodja T, Kadanga MM, Bara FD, Simfele HC, Kolou M, Mbengue B. Optimizing whole blood stimulation conditions for intracellular cytokine staining: A solution for resource-limited laboratories and field studies. *WoSciMIB*. 2025; 01(04):1-8. DOI: **10.5281/zenodo.15318008**

Abstract

Introduction: Intracellular cytokine staining (ICS) combined with flow cytometry is a preferred method for evaluating and characterizing cell-mediated immune responses. While peripheral blood mononuclear cells (PBMCs) are commonly used in immunological studies, their isolation poses challenges in remote or resource-limited settings. This study aimed to establish optimal conditions for whole blood stimulation to assess cytokine-producing T cells, specifically interferon gamma (IFN-γ), interleukin-17A (IL-17A), and interleukin-10 (IL-10).
Methods: Eight healthy male donors, aged 28–35 years, participated in this study. Of these, 3 donors were included to assess $CD4^+Tbet^+IFN\gamma^+$, $CD4^+GATA\text{-}3^+IL\text{-}4^+$, $CD4^+ROR\gamma t^+IL\text{-}17A^+$, and $CD4^+FOXP\text{-}3^+IL\text{-}10^+$ cells using whole blood samples and PBMCs from the same donors and fives were recruited to investigate only the effects of varying whole blood samples volumes and different container types assessing only $CD4^+Tbet^+IFN\gamma^+$. Cells were identified through flow cytometry after stimulation of PBMCs and whole blood samples using phorbol 12-myristate 13-acetate (PMA) and ionomycin.
Results: This study has two main limitations including the very small sample size and the cell culture plate which did not allow stimulation at 200 µL of whole blood. However, the results remain debatable with the use of Falcon tubes. The percentage of $CD4^+Tbet^+IFN\text{-}\gamma^+$ cells did not differ significantly when a cell culture plate was compared to a Falcon®-type tube using whole blood sample. However, cytokine response was significantly higher when 100 µL of whole blood was used in cell culture plates compared to 200 µL in Falcon®-type tubes.
Conclusion: This study suggests that 100 µL of whole blood is a practical alternative to PBMCs, with 96-well plates serving as the optimal container for T-cell stimulation in resource-limited settings.

Keywords: Whole blood sample, Intracellular cytokine staining, Flow cytometry, T cells

Introduction

T cells play an essential role in the specific immune response. These lymphocytes, derived from the thymus, can be further categorized into subpopulations based on their phenotypic markers and the cytokines they express, which reflect their functional roles (1). Numerous diagnostic methods are designed to study T cells as well as the pathologies associated to their dysfunctions. Among the various methods available for analyzing T-lymphocyte functions, intracellular cytokine staining (ICS) by flow cytometry is considered as the gold standard (2). This approach not only detects cytokine expression at the single-cell level but also enables the simultaneous detection, quantification, and phenotypic characterization of T cells (3). ICS can be performed on Peripheral Blood Mononuclear Cells (PBMCs) (4) or directly on Whole Blood (WB) (5).

PBMCs are typically isolated from whole blood using Ficoll density gradient centrifugation allowing for stimulation, staining and immediate analysis or cryopreservation. However, the isolation process is labor-intensive, time-consuming, and expensive, involving lengthy centrifugation steps that can be challenging in settings with unreliable electricity. These constraints make PBMC isolation less practical in low-income countries. Additionally, PBMC isolation often requires large blood volumes, which may not be ethically acceptable or culturally appropriate in certain populations, such as pediatric patients or pregnant women (6-9).

In contrast, whole blood offers a more practical alternative. It retains all host serum factors and cellular components, providing a more physiological environment for stimulation by maintaining cell-to-cell communication (10). Whole blood procedures require minimal material, fewer preprocessing steps, and only small blood volumes, which is advantageous in resource-limited settings. However, a key challenge with whole blood lies in determining the number of cells available for stimulation, as this may vary between individuals and depends on the blood volume used. Although, the use of whole blood sample does not solve the electricity problem in low-income countries, it does solve the budgetary constraints, ethical and societal problems related to sample collection.

Recent studies have highlighted the potential of whole blood for analyzing cellular functionality using ICS and flow cytometry. Nevertheless, critical aspects such as the optimal blood volume and type of cell culture vessel for studying cytokine-producing T cells remain poorly understood.

This study aims to optimize whole blood stimulation conditions for intracellular cytokine staining, focusing on cytokine-producing T cells. We investigated the expression of key cytokines, including interferon gamma (IFN-γ), interleukin-4 (IL-4), IL-17A, IL-10 in $CD4^+$ T lymphocytes. Furthermore, we assessed the use of 15 ml Falcon-type tubes and 96-well U-bottom plates to establish optimal stimulation conditions using varying volumes of whole blood samples.

Methods

Study population

This descriptive, cross-sectional study was conducted in two stages with a total of eight (08) male healthy donors aged 28 - 35 years recruited at the blood collection and distribution point of Centre Hospitalier Universitaire Sylvanus Olympio (CHU SO). Firstly, three healthy donors were included to assess cytokines producing T cells using whole blood samples and PBMCs from the same donors. Secondly, fives donors were recruited to investigate only the effects of varying whole blood volumes and different container types. Venous blood samples were collected from each participant in ethylene diamine tetra acetic acid (EDTA) tubes and immediately transported to the Unité de Recherche en Immunologie et Immuno-modulation (UR2IM) /Laboratoire de Microbiologie et de Contrôle Qualité des Denrées Alimentaires (LAMICODA)/ Université de Lomé, Togo for cellular analyses. All protocols were started within the next two hours after blood collection on the same day.

Immunophenotyping of T cell subpopulations

Peripheral Blood Mononuclear Cells (PBMCs) isolation and stimulation: PBMCs from fresh whole blood samples were isolated using the Ficoll Paque (PAN-Biotech) density gradient centrifugation method on ice as described by Simfele et al. (11). The cells were suspended in culture medium (Roswell Park Memorial Institute 1640 supplemented with gentamicin 50 µg/mL, penicillin streptomycin 100 µg/mL, L-glutamine 2 mM/mL ($RPMI^{+++}$), and fetal bovine serum (FBS) at 10% (PAN-Biotech) at the concentration of 2×10^5 cells/µL and then the cells were counted and their viability assessed by Trypan blue exclusion method. A One hundred microliters (100 µL) of PBMCs suspension per well was stimulated in duplicate as described by Tchopba et al. (12) using 96- wells U-bottom plates (Greiner Bio One, Frickenhausen, Germany) with 50 µL of 1X cell stimulation cocktail (phorbol 12-myristate 13-acetate (PMA), Ionomycin, Brefeldin A and Monensin; eBioscience), and incubated at 37°C in presence of 5% CO_2 for 6 hours. Thereafter, cells were harvested and wash before staining.

Whole blood stimulation: Fresh whole blood was cultured in RPMI complete medium ($RPMI^{+++}$ and fetal bovine serum (FBS) at 10%), in 96- wells U-bottom plates (Greiner Bio One, Frickenhausen, Germany) and 15 ml polypropylene conical bottom (Greiner Bio One,

Frickenhausen, Germany) under sterile conditions. Different volumes of whole blood were tested in 15 mL Falcon® tubes to determine the optimal stimulation volume. Equal volumes of EDTA-treated whole blood were mixed with RPMI-1640 medium and 1X cell stimulation cocktail in duplicate as follow: for 100 µL blood samples we add 100 µL of 1X cell stimulation cocktail with RPMI-1640 medium and for 200 µL blood samples, 200 µL of 1X cell stimulation cocktail with RPMI-1640 medium were added. All tubes incubations were conducted as described above. After stimulation, red blood cells were lysed from stimulated blood samples using RBC Lysis Buffer (Roche® Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. After that, cells were stained under the same conditions with PBMCs.

Cells staining and gating strategy: To assess cytokine expression in $CD4^+$T cells, cell-surface staining was performed using APC-conjugated anti-CD4 (clone A161A1) antibodies (1:50) and incubated at 4°C for 30 minutes.

Intracellular staining followed, using Fix-Perm (eBioscience) reagent for fixation and permeabilization and Fc block (human TruStain FcX®, BioLegend, San diego, CA, USA) to prevent non-specific binding. Four staining panels were used: 1. Anti-human T-bet-PE (clone 4B10) and Anti-human IFN-γ-FITC (clone 4S.B3); 2. Anti-human GATA3-PE (clone 16E10A23) and Anti-human IL-4-FITC (clone MP4-25D2); 3. Anti-human RORγt-PE (clone AFKJS-9) and Anti-human IL-17A-FITC (clone BL168); 4. Anti-human FoxP3-FITC (clone 206D) and Anti-human IL-10-PE (clone JES3-9D7). Samples were washed, resuspended in fix-perm buffer, and analyzed using a flow cytometer (Cytoflex, Beckman Coulter, Brea, California, USA). Data were acquired using CytExpert 2.1 software (Cytoflex, Beckman Coulter, Brea, California, USA), and gating strategies were used to identify $CD4^+$ T-cell subpopulations from PBMCs (Fig. 1) and whole blood samples (Fig. 2). Unstained and single-stained cells served as negative and positive controls, respectively. All antibodies were obtained from BioLegend (BioLegend®CA, USA).

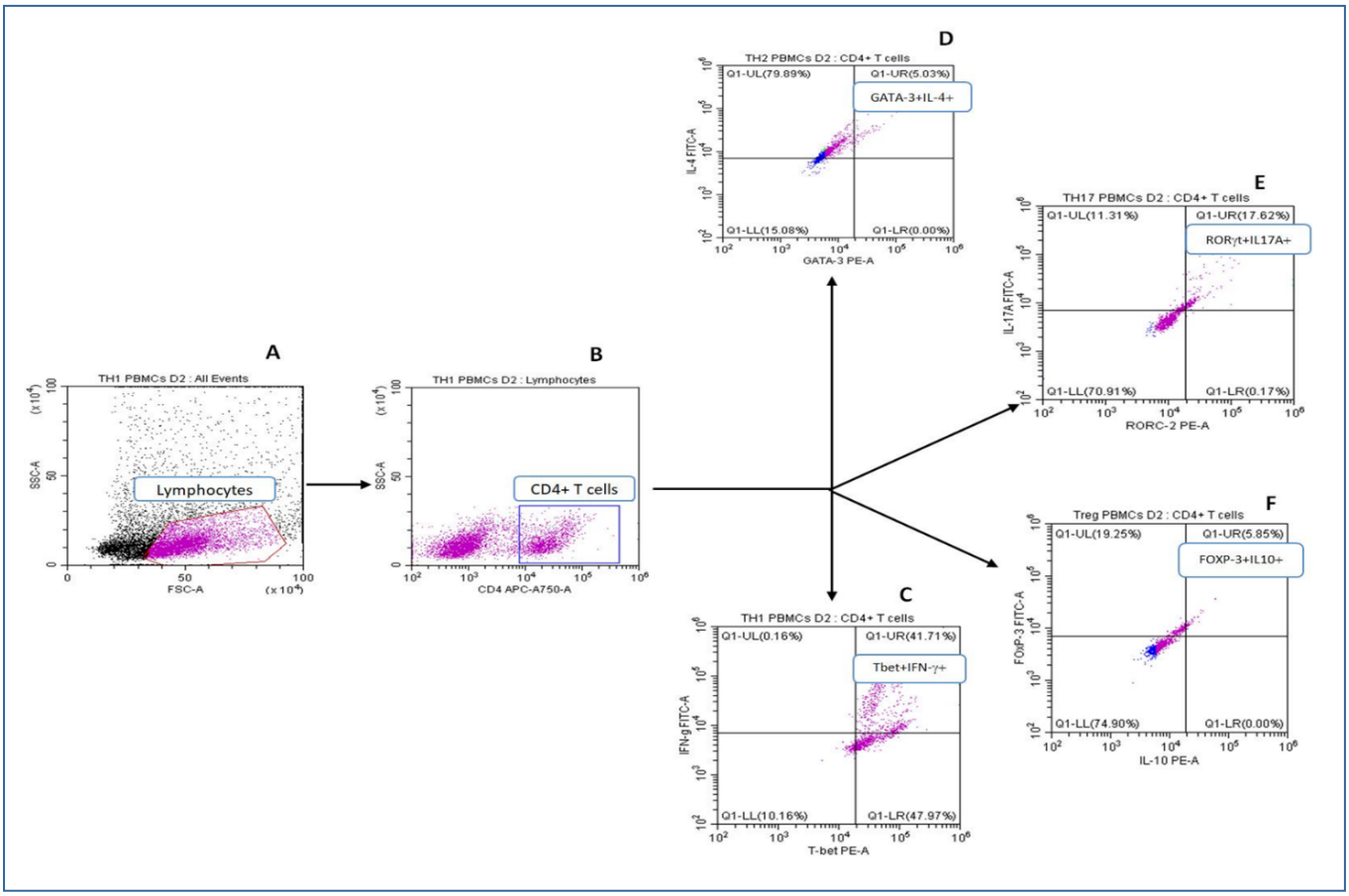

Fig. 1: Gating strategy for assessing Cytokine producing T-cells from PBMCs. Lymphocytes gate (total T cells) were set based on cells granularity and size defined by Forward scatter (FSC) and side scatter (SSC) (1A) then from them, we identified $CD4^+$T-cells (1B) which were then separated into different subpopulation $CD4^+Tbet^+IFN\gamma^+$ (1C), $CD4^+GATA\text{-}3^+IL\text{-}4^+$ (1D), $CD4^+ROR\gamma t^+IL\text{-}17A^+$ **(**1E), and $CD4^+FOXP\text{-}3^+IL\text{-}10^+$ (1F).

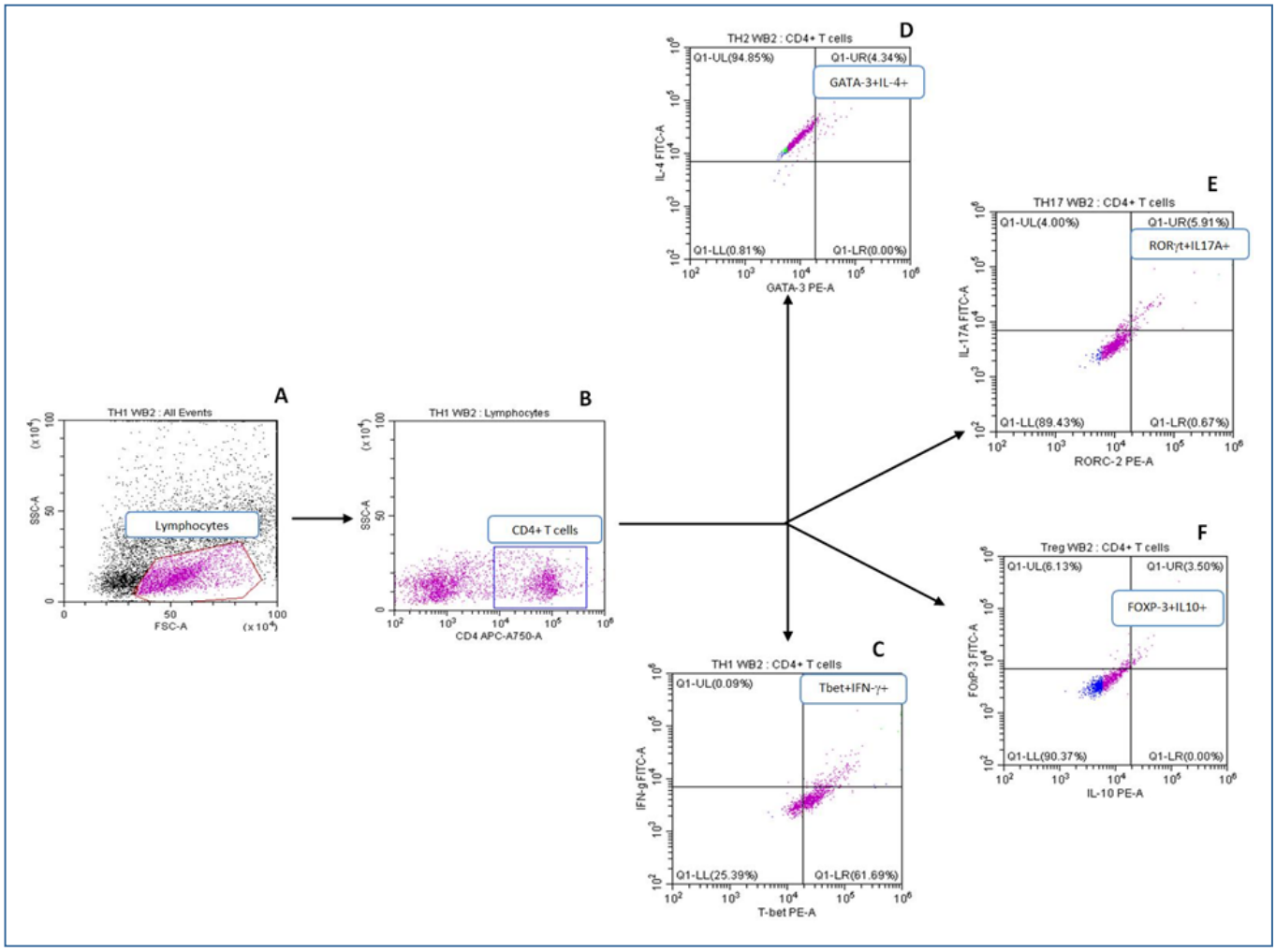

Fig. 2: Gating strategy for assessing Cytokine producing T-cells from whole blood. Lymphocytes gate (total T cells) were set based on cells granularity and size defined by Forward scatter (FSC) and side scatter (2A) then from them, we identified $CD4^+$T-cells (2B) which were then separated into different subpopulation $CD4^+Tbet^+IFN\gamma^+$ (2C), $CD4^+GATA\text{-}3^+IL\text{-}4^+$ (2D), $CD4^+ROR\gamma t^+IL\text{-}17A^+$ (2E), and $CD4^+FOXP\text{-}3^+IL\text{-}10^+$ (2F).

Statistical analysis

Data were compiled in Excel and analyzed using GraphPad Prism version 5.02 (GraphPad Software, La Jolla, CA, USA). Differences between groups were assessed using non-parametric Mann-Whitney U tests with statistical significance set at $p < 0.05$.

Ethics statement

Ethical approval for this study was obtained from the bioethics committee for health research of Togo ("Comité de Bioéthique pour la Recherche en Santé", CBRS) under approval number 039/2024/CBRS. Written informed consent was obtained from all participants prior to sample collection.

Results

Comparison of cytokines-producing T cells between whole blood and PBMCs

We compared the frequencies of cytokine-producing T cells, including $CD4^+Tbet^+IFN\gamma^+$(Th1), $CD4^+GATA3^+IL\text{-}4^+$ (Th2), $CD4^+ROR\gamma t^+IL\text{-}17A^+$ (Th17) and $CD4^+FoxP3^+IL\text{-}10^+$ (Treg), between whole blood and PBMCs as shown in Fig 2 and summarized in Table 1. Our findings revealed that Th1 cells were significantly more abundant in PBMCs compared to whole blood (Fig. 3A). In contrast, no significant differences were observed for Th2 (Fig. 3B), Th17 (Fig. 3C), or Treg cells (Fig. 3D), Although, all these T cells subsets were higher in PBMCs than in whole blood. Additionally, the standard deviation (SD) was notably larger for PBMCs

than for whole blood, indicating greater variability in cell frequencies between individuals when PBMCs were used.

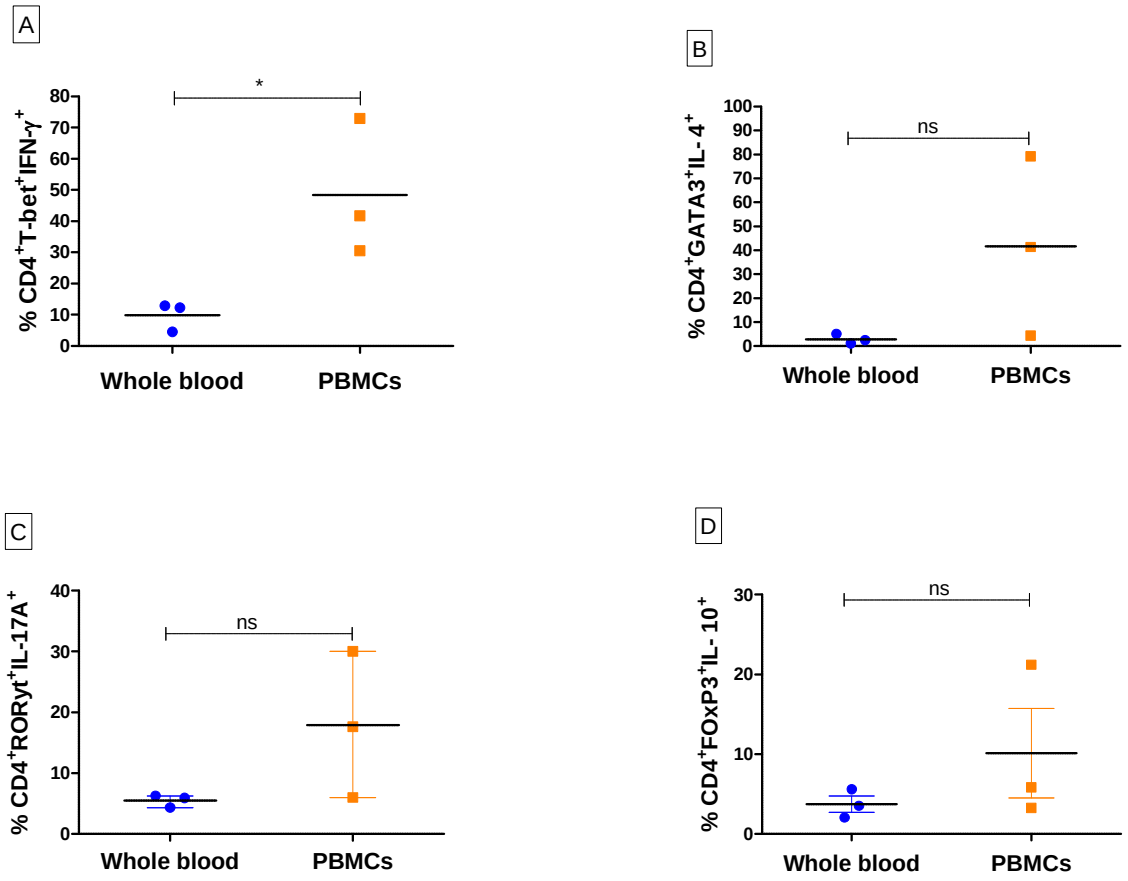

Fig. 3: Percentage of T cells cytokines-expression in whole blood samples and PBMCs (n=3) stimulated with PMA, Ionomycin, Brefeldin A and Monensin. *, p < 0.05; ns, non-significant. The Mann-Whitney U test was used to compare differences among groups. A: Th1 ($CD4^+Tbet^+IFN\gamma^+$); B: Th2 ($CD4^+GATA3^+IL\text{-}4^+$); C: Th17 ($CD4^+ROR\gamma t^+IL\text{-}17A^+$); D: Treg ($CD4^+FoxP3^+IL\text{-}10^+$). Scatter plots show the mean and standard error of the mean (SEM). Mann–Whitney U test was used for statistical comparisons. Each dot represents an individual data point, and the horizontal black lines represent the mean.

Table I: Variability in cytokine-producing T cells between whole blood and PBMCs, expressed as mean. Standard deviation (SD) reflects the variability around the mean.

Cell type	Whole Blood (n=3)	PBMCs (n=3)	p-value
	Mean (SD)	Mean (SD)	
$CD4^+Tbet^+IFN\gamma^+$	9.85 (4.67)	48.37 (21.96)	**0.0500**
$CD4^+GATA3^+IL\text{-}4^+$	2.87 (1.98)	41.66 (37.45)	0.1000
$CD4^+ROR\gamma t^+IL\text{-}17A^+$	5.49 (1.03)	17.88 (12.02)	0.1000
$CD4^+FoxP3^+IL\text{-}10^+$	3.71 (1.78)	10.11 (9.70)	0.2000

Effect of Falcon® tube used for stimulation on T cells response

Since the significant difference was observed with the $CD4^+Tbet^+IFN\gamma^+$ cells and due to budgetary constraints, this subtype was chosen to examine whether the type of plastic container used for stimulation influences cytokine-producing T cell expression. As shown in Fig. 4E, no significant difference was observed in the percentage of $CD4^+Tbet^+IFN\gamma^+$ cells when comparing stimulations performed in cell culture plates versus Falcon® tubes with 100 µL of whole blood.

Impact of volume of whole blood samples stimulated

The effect of increasing the volume of whole blood used for stimulation was also assessed. While an increase to 200 µL resulted in a lower response, the difference was not statistically significant when using Falcon® tubes (Fig. 4F). Interestingly, when 100 µL of whole blood was used in a cell culture plate, the response was significantly higher compared to 200 µL in a Falcon® tube.

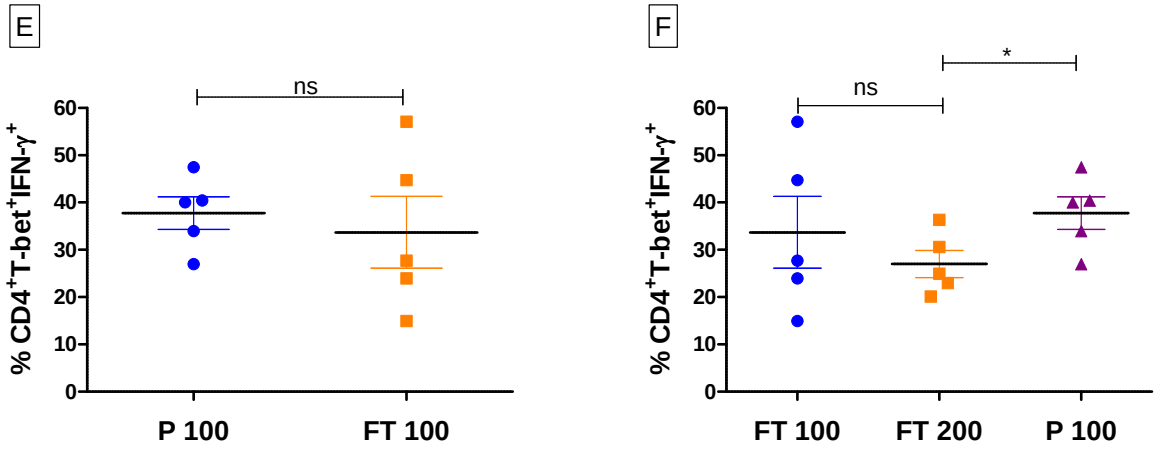

Fig. 4: Impact of plastic container type on the percentage of CD4+Tbet+IFNγ + (n=5). (E) Comparison of cell culture plates with 100 µL of whole blood (P 100) and Falcon® tubes with 100 µL of whole blood (FT 100). (F) Comparison of plastic containers (Falcon® tubes vs. plates) and blood volumes (100 µL vs. 200 µL). *p < 0.05; ns, not significant; Falcon® tubes with 100 µL of whole blood (FT 200). Each dot represents an individual data point, and the horizontal black lines represent the mean. Statistical analysis was performed using the Mann–Whitney U test.

Discussion

Whole blood stimulation assays are widely utilized in clinical research, for applications such as disease characterization, evaluating host immunity, diagnosis testing, treatment monitoring, and drug development (13). Compared to PBMCs, whole blood is more readily accessible in clinical settings, making it a potentially convenient alternative for studying immune responses. This study aimed to optimize the conditions under which whole blood could serve as an alternative to PBMCs for assessing T cell responses using intracellular cytokine staining (ICS) by flow cytometry. Specifically, we sought to determine the optimal volume of whole blood and the most suitable cell culture containers for stimulation.

Our study included firstly three healthy male donors, with both whole blood and PBMCs stimulated under identical conditions for comparison building on previous studies of cell-mediated responses using intracellular cytokine staining (ICS) in PBMCs and whole blood (2).

One hundred microliters (100 µL) of whole blood and PBMCs were used to compare the cytokine-producing T cell subpopulations. For surface marker labeling, We chose to perform staining after red blood cell (RBC) lysis (14), as this method seemed more appropriate for our experimental setup. Although some studies advocate staining before lysis (15,16). The results highlighted inter-individual variability in responses, which was more pronounced when PBMCs were used compared to whole blood. According to Moris et al., (2) who reported highly consistent CD4+ T cell responses between whole blood and PBMCs in their clinical trials, these differences observed in our study could stem from variations in cell concentration (unknown cells number or concentration, smaller volume of whole blood used) or

differences in stimulation conditions. As whole blood stimulation is closest to in vivo setting compared to peripheral blood mononuclear cells (13). Moreover, the non-significant differences observed in $CD4^+GATA3^+IL\text{-}4^+$; $CD4^+ROR\gamma t^+IL\text{-}17A^+$; $CD4^+FoxP3^+IL\text{-}10^+$ cells despite being higher in PBMcs could be related to our sample size which was too small due to lack of resources.

To explore the hypothesis that whole blood volume stimulated influences in vitro responses, fives donors were included to investigate the effects of varying whole blood volumes and different container types (Falcon tubes vs. 96-well U bottom plates) on the stimulation results focusing on Th1 (CD4+Tbet+IFNγ+) cells, as this population showed statistically significant differences between whole blood and PBMC assays.

Our findings revealed no significant difference in Th1 cell percentages when comparing stimulation in cell culture plates to Falcon tubes, suggesting that Falcon tubes can serve as viable alternatives for cell stimulation (3). However, increasing the volume of whole blood from 100 µL to 200 µL led to a decrease in response, although this difference was not statistically significant. Since the 96-well U bottom plates could not hold the total volume of whole blood sample including RPMI complete medium and stimulant (PMA) with 200 µL whole blood, we first compared the plate versus Falcon tube with 100 µL blood volume. The results showed no significant difference, so we assumed to compare the plate with different blood volumes using the Falcon tube.

Conversely, stimulation with 100 µL of whole blood in 96-well U bottom plates resulted in significantly higher Th1 responses compared to 200 µL in Falcon tubes. These results indicate that using 100 µL of whole blood in cell culture plates provides the optimal condition for characterizing T cell responses through ICS.

Measurements of vaccine immunogenicity in preclinical development and clinical trials routinely include quantification of antigen-specific Th1 and Th17 cells by ICS, and ELISA-based quantification of cytokines-release (17). Thus, this conclusive evaluation took into account the cellular aspect (Th1, Th2, Th17 and regulatory T cells) and the ICS to be applicable. In addition, it will constitute a basis for its applicability for the measurement of immunological markers with prognostic and diagnostic values in clinical biology. However, our study has some limitations. The small sample size may limit the generalizability of our results, and further studies should include larger cohorts with diverse population in age, sex and even their immune status and additional cell types to validate our observations. Additionally, the use of PMA and Ionomycin as activating stimuli may have affected CD4 expression levels (18), potentially leading to artificially lower proportions of cytokine-producing CD4+ T cells. Alternative stimulation protocols or adjustments to the current method could address this limitation in subsequent experiments.

Conclusion

This study suggests that 100 µL of whole blood assays can serve as a practical and reliable alternative to PBMC-based assays, for assessing T cell responses, with 100 µL of whole blood in 96-well cell culture plates being the optimal conditions for T-cell stimulation in resource-limited settings. These findings provide a foundation for further research to refine whole blood-based assays, making them more accessible and practical for a range of clinical and research applications.

Conflict of interest

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article

Author's contribution

Gnodja Tégmaba: Conceived and designed the study, enrolment, performed the experiments, formal analysis, writing original draft, participated in writing-review and editing.

Kadanga Motomdéwa Monique: Performed the experiments, formal analysis, participated in writing-review and editing.

Bara Fagdéba David: Performed the experiments, formal analysis, participated in writing-review and editing.

Simfele Hombamane Christelle: Performed the experiments, formal analysis, participated in writing-review and editing.

Kolou Maléwé: Conceived and designed the study, participated in writing-review and editing, contributed reagents/materials, supervision and validation.

Mbengue Babacar: Conceived and designed the study, participated in writing-review and editing, contributed reagents/materials, supervision and validation.

References

1. Maecker HT. Cytokine flow cytometry. Methods Mol Biol. 2004;263:95–108.

2. Moris P, Bellanger A, Ofori-Anyinam O, Jongert E, Yarzabal Rodriguez JP, Janssens M. Whole blood can be used as an alternative to isolated peripheral blood

mononuclear cells to measure in vitro specific T-cell responses in human samples. Journal of Immunological Methods. 2021 May 1;492:112940.

3. Mandala W, Harawa V, Munyenyembe A, Soko M, Longwe H. Optimization of stimulation and staining conditions for intracellular cytokine staining (ICS) for determination of cytokine-producing T cells and monocytes. Current Research in Immunology. 2021;2:184–93.

4. Waldrop SL, Pitcher CJ, Peterson DM, Maino VC, Picker LJ. Determination of antigen-specific memory/effector CD4+ T cell frequencies by flow cytometry: evidence for a novel, antigen-specific homeostatic mechanism in HIV-associated immunodeficiency. J Clin Invest. 1997 Apr 1;99(7):1739–50.

5. Suni MA, Picker LJ, Maino VC. Detection of antigen-specific T cell cytokine expression in whole blood by flow cytometry. J Immunol Methods. 1998 Mar 1;212(1):89–98.

6. Howie SRC. Blood sample volumes in child health research: review of safe limits. Bull World Health Organ. 2011 Jan 1;89(1):46–53.

7. Germann A, Oh YJ, Schmidt T, Schön U, Zimmermann H, Von Briesen H. Temperature fluctuations during deep temperature cryopreservation reduce PBMC recovery, viability and T-cell function. Cryobiology. 2013 Oct;67(2):193–200.

8. Boahen O, Owusu-Agyei S, Febir LG, Tawiah C, Tawiah T, Afari S, et al. Community perception and beliefs about blood draw for clinical research in Ghana. Transactions of The Royal Society of Tropical Medicine and Hygiene. 2013 Apr 1;107(4):261–5.

9. Peplow C, Assfalg R, Beyerlein A, Hasford J, Bonifacio E, Ziegler AG. Blood draws up to 3% of blood volume in clinical trials are safe in children. Acta Paediatrica. 2019;108(5):940–4.

10. Brodin P, Duffy D, Quintana-Murci L. A Call for Blood-In Human Immunology. Immunity. 2019 Jun 18;50(6):1335–6.

11. Simfele HC, Nadjir KL, Kolou M, Katawa G. Flow cytometry technique for identification and functional analysis of NK cells. 2024 Sep 17 [cited 2024 Dec 17]; Available from: https://zenodo.org/doi/10.5281/zenodo.13767356

12. Tchopba CN, Katawa G, Padaro E, Tchadié PE, Karou SD, Vovor A, et al. Systemic T Cell Subsets and Cytokines in Patients With Homozygous Sickle Cell Disease and Asymptomatic Urinary Tract Infections in Togo. Ochsner J. 2021;21(2):163–72.

13. Müller S, Kröger C, Schultze JL, Aschenbrenner AC. Whole blood stimulation as a tool for studying the human immune system. European Journal of Immunology. 2024;54(2):2350519.

14. Cepika AM, Banchereau R, Segura E, Ohouo M, Cantarel B, Goller K, et al. A multidimensional blood timulation assay reveals immune alterations underlying systemic juvenile idiopathic arthritis. J Exp Med. 2017 Nov 6;214(11):3449–66.

15. Basso-Ricci L, Scala S, Milani R, Migliavacca M, Rovelli A, Bernardo ME, et al. Multiparametric Whole Blood Dissection: A one-shot comprehensive picture of the human hematopoietic system. Cytometry A. 2017 Oct;91(10):952–65.

16. De Domenico E, Bonaguro L, Schulte-Schrepping J, Becker M, Händler K, Schultze JL. Optimized workflow for single-cell transcriptomics on infectious diseases including COVID-19. STAR Protoc. 2020 Dec 18;1(3):100233.

17. Abel B, Tameris M, Mansoor N, Gelderbloem S, Hughes J, Abrahams D, et al. The novel tuberculosis vaccine, AERAS-402, induces robust and polyfunctional CD4+ and CD8+ T cells in adults. Am J Respir Crit Care Med. 2010 Jun 15;181(12):1407–17.

18. Godoy-Ramirez K, Franck K, Mahdavifar S, Andersson L, Gaines H. Optimum culture conditions for specific and nonspecific activation of whole blood and PBMC for intracellular cytokine assessment by flow cytometry. Journal of Immunological Methods. 2004 Sep;292(1–2):1–15.