Submitted 1.24.11 | Revision Received 2.16.11 | Accepted 2.24.11

Setting Up a Standardized Peripheral Blood Mononuclear Cells Processing Laboratory to Support Multi-center HIV/AIDS Vaccine and Intervention Trials

Harr Freeya Njai, PhD,[1] Ben Gombe, BSc,[1] Tomusange Khamis, BSc,[1] Josephine Birungi, PhD,[2] Eugene Ruzagira, MD,[1] Dareskedar Admassu, MSc,[3] Tony Tarragona-Fiol, PhD,[3] Kholoud Porter, PhD,[4] Gwynneth Stevens, PhD,[5] Joseph Mugisha, MD,[1] Jill Gilmour, PhD,[3] Anatoli Kamali, MD, MPH,[1] Pontiano Kaleebu, MD, PhD[1,2]

([1]MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda, [2]UVRI-IAVI HIV Vaccine Program, Entebbe, Uganda, [3]International AIDS Vaccine Initiative [IAVI] Core Laboratory, Imperial College London, London, United Kingdom, [4]MRC Clinical Trial Unit, London, United Kingdom, [5]International AIDS Vaccine Initiative (IAVI), Johannesburg, Gauteng, South Africa)

DOI: 10.1309/LM84WWEUSKT4ABXO

Abstract

Despite infrastructure and capacity challenges in Africa, significant development has been made in the number of laboratories supporting immunological and safety studies required for large-scale HIV/AIDS vaccine or intervention trials. In Uganda, cohorts participating in HIV intervention trials are often recruited from rural areas. To avoid transporting samples from intervention trial areas over long distances (120 km) to central laboratories in Entebbe, we set up a standardized peripheral blood mononuclear cells (PBMCs) processing laboratory at a field station in Masaka, southwest Uganda. The laboratory was well equipped and enrolled into the International AIDS Vaccine Initiative (IAVI) Quality Assurance (QA) program. Staff was trained in laboratory techniques and Good Clinical Laboratory Practice (GCLP). The laboratory received IAVI and GCLP accreditation in 2008. In this paper we describe the process and achievements of measures taken to overcome challenges, to build staff capacity, and to optimize the quality of the cells yielded.

Keywords: Good Clinical Laboratory Practice, standardized laboratory procedures, capacity building, rural Africa, HIV/AIDS vaccine or intervention trials

HIV vaccine safety and efficacy trials will often involve investigations of the cellular immune system of study participants, including HIV-1-specific CD8 cytotoxic T lymphocytes (CTL) and CD4 T helper (Th) lymphocytes. In order to achieve good and valid results, the collection and processing of fresh and cryopreserved peripheral blood mononuclear cells (PMBCs) must be optimal. The quality of the resulting PBMC may be influenced by a number of the following factors: blood collection,[1-3] processing,[4] and shipping.[5] Recently it was shown that the length of time from venipuncture to cryopreservation is the single most important parameter influencing the quality of cells,[6] and processing should occur within 8 hours of venipuncture. This narrow window for specimen processing has important implications in selecting and monitoring clinical sites with laboratory capacity to perform these procedures in future clinical trials.

The use of fresh PBMC cells is preferred,[5-7] but cryopreserved PBMC has advantages. It allows researchers to conduct batch testing, and cryopreserved PBMCs can also be used after the conclusion of a clinical trial to help aid the interpretation of findings and if questions arise due to new developments in the field. Most important, it allows participation of remote sites in African countries that are part of multi-center studies, and indeed, laboratories in Africa are increasingly being used to support internationally initiated clinical research trials for HIV prevention and vaccine development.[8]

Corresponding Author

Harr Freeya Njai, PhD
harrnjai@yahoo.com

Abbreviations

PBMCs, peripheral blood mononuclear cells; IAVI, International AIDS Vaccine Initiative; QA, quality assurance; GCLP, Good Clinical Laboratory Practice; CTL, cytotoxic T lymphocytes; Th, T helper; UVRI, Uganda Virus Research Institute; SOPs, standard operating procedures; SPARTAC, short pulse anti-retroviral treatment at seroconversion; S101, sample 1, operator 1; S102, sample 1, operator 2; S201, sample 2, operator 1; S202, sample 2, operator 2; ELISPOT, enzyme-linked immunosorbent spot; CLS, Clinical Support laboratory; DAIDS, division of AIDS

The importance of building and maintaining laboratory capacity to support global health systems was the central focus of a recent conference in Kigali.[9-14] In Africa, study populations are often located at substantial distances from centers in which highly standardized laboratories exist. The MRC/UVRI Uganda Research Unit on AIDS has a center of excellence laboratory with Good Clinical Laboratory Practice (GCLP) accreditation, 120 km away from Masaka, southwest Uganda; 1 of the study sites. However, road conditions and weather seasons pose additional challenges for optimal sample transportation and delivery. It would therefore be highly beneficial to have well-functioning PBMC labs established that are situated close to study populations. The establishment of a standardized, high-quality PBMC processing laboratory in comparatively remote locations is in itself associated with major challenges: difficulties around reagent procurement, purchase delivery times, equipment servicing, unstable electrical supply, and inconsistent liquid nitrogen supply are common. Other major challenges are related to personnel training and to safety issues such as the effective disposal of waste and toxic reagents, prevention of fires, and the exposure of staff to biohazardous substances.

This paper describes a number of simple measures taken to establish a sustainable, well-functioning PBMC processing lab in a field site in Africa. We also report on the results of the quality of tests performed by this laboratory. We would like to share this experience as it may be helpful to other research groups working on immunological studies in Africa.

Methods

I. Infrastructure Development

The Masaka Cell Laboratory consists of 2 adjoining rooms, renovated to a standard laboratory space. The laboratory was then equipped with a class II biosafety cabinet, a refrigerated centrifuge, a +37°C water bath, a light microscope, a +4°C/-20°C refrigerator/freezer, -80°C freezers, an ice maker, a control rate freezer, and liquid nitrogen freezing tanks. Uninterrupted power supply sources were secured and installed with all equipment, as well as a back-up generator to ensure a constant power supply. We established the procurement of a constant liquid nitrogen supply, in addition to a back-up liquid nitrogen tank (30 L) maintained for an emergency. Most importantly, a disaster recovery policy was developed and implemented to ensure that a back-up GCLP-accredited lab was identified nearby, with the capacity to provide liquid nitrogen and to store cell samples.

II. Personnel Recruitment, Training, and Securing Laboratory Supplies

A virologist with a PhD in biological sciences led the team in setting up the laboratory. The virologist had post-doctoral experience in immunology, with a focus on HIV/AIDS, in addition to experience living and working in Africa. Two laboratory technologists were also recruited after placing advertisements in local Ugandan newspapers. Successful applicants had first degrees (BSc) in biomedical laboratory technology.

A significant investment was made in the training of laboratory staff technologists, composed of: 1) PBMC processing, 2) storage, and 3) shipping procedures. Uganda Virus Research Institute (UVRI)- International AIDS Vaccine Initiative (IAVI) Vaccine Program laboratory in Entebbe, a GCLP-accredited lab, conducted staff training on various techniques using standardized UVRI-IAVI site-specific and IAVI core laboratory standard operating procedures (SOPs). At the end of the training, technologists were subjected to proficiency testing. The IAVI trained laboratory management in procurement, budgeting, cost of tests, shipping, preventive maintenance of equipment, the management of logistics, and staff skills. To ensure continuous availability of laboratory supplies in Masaka, the MRC/UVRI's established procurement system was adopted. Adequate storage facilities were provided to keep working stocks in the Masaka laboratory and long-term storage in the Entebbe laboratory. Masaka is 120 km from Entebbe. The separation of stock storage is a disaster recovery strategy.

III. Guidelines and Policies

Good Clinical Laboratory Practice compliance is the minimal requirement clinical laboratories should meet to increase adherence to standardized practices and procedures, optimize management operations of clinical laboratories, and enhance obtaining reproducible and reliable results, while ensuring the safety of human research participants.[15-18] All laboratory technologists were trained in basic concepts and advanced GCLP and laboratory management. Site-specific guidelines and policies were developed to facilitate the implementation of GCLP, electricity back-up plans, sustainable liquid nitrogen supply, disaster recovery, and safety. Twenty-one site-specific SOPs were developed and implemented, covering 4 major areas: 1) management, 2) sample handling, 3) quality control, and 4) health and safety.

IV. Laboratory Assays

Standardized operating procedures on PBMC processing were introduced. At our site, we currently use SOPs from 2 international multi-center studies: The IAVI-sponsored Acute Infection Study and the Wellcome Trust funded Short Pulse Anti-Retroviral Treatment at Seroconversion (SPARTAC) Trial. The Masaka Cell Laboratory was then enrolled into the IAVI Quality Assurance (QA) program; this involves individual competency (dry-run experiments), internal control (quality control), external assurance features (qualifying-run experiments), and independent audits.

Results

I. Dry-run Experiments

Peripheral blood mononuclear cells from 2 blood packs were isolated and cryopreserved. To analyze inter-operator variability, each donor blood pack was divided into 2 equivalent aliquots, which were handled independently by the 2 technologists at the Masaka Cell Laboratory. All PBMCs were isolated using modified Ficoll density centrifugation according to study protocol. A mean PBMC yield of 1.17×10^6 cells/ml and >98% viability was observed (expected yield of 0.8 - 3.0 $\times 10^6$ cells/mL). Between operators we observed comparable PBMC yields. In **Figure 1**: sample 1 (S1), processed by operator 1 (S1O1), had a yield of 1.3×10^6 cells/mL as compared to the same sample processed by operator 2 (S1O2), with a yield of 1.2×10^6 cells/mL. Using another sample (S2), similar yields were observed from both operators: 1.1×10^6 cells/mL (S2O1) and 1.07×10^6 cells/mL (S2O2). Replicate cryopreserved vials were then sent to the UVRI-IAVI HIV Vaccine Program laboratory in Entebbe, where they were thawed, rested overnight, and analyzed by 2 operators independently

Image 1_Papyrus field on the Entebbe - Masaka Road.

Image 2_MRC/UVRI Uganda Research Unit on AIDS, Masaka Vaccine Trial Clinic.

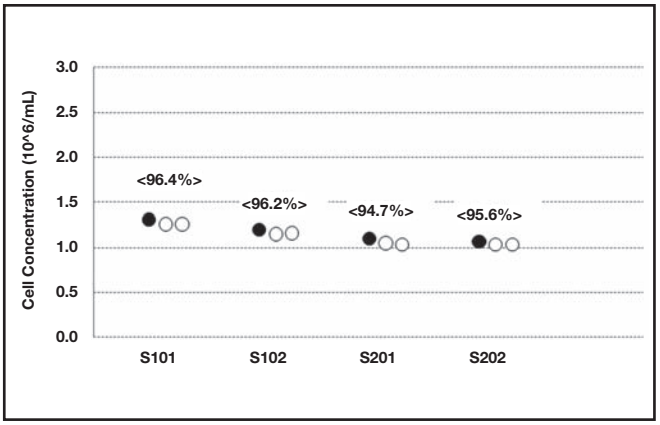

Figure 1_Dry Run: PBMC processing from whole blood. Graph indicates PBMC yields from 2 blood packs processed by 2 operators simultaneously with an average yield of 1.17 × 10^6 cells/mL. Solid black circles indicate the PBMC yield on the day of the processing. Clear circles indicate recovery of stored samples in duplicate. The acceptance criteria for frozen cell recovery and viability are: at Day 1: % recovery >70% and % viability >80%; at Day 2: % recovery >60% and % viability >70%. Recovery of all 4 samples was >94%, well above the QA acceptance criteria of 60%. S101, sample 1, operator 1; S102, sample 1, operator 2; S201, sample 2, operator 1; S202, sample 2, operator 2.

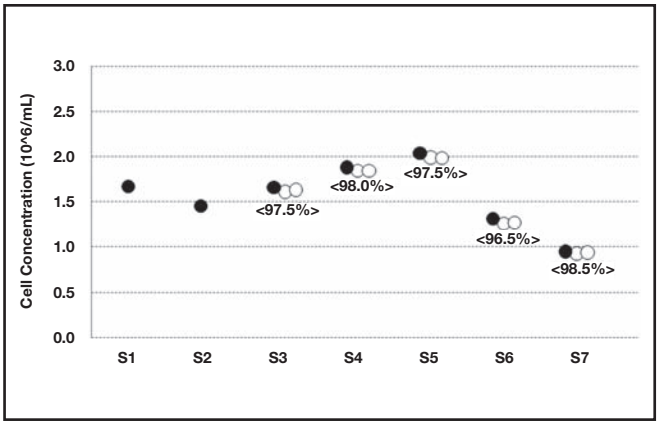

Figure 2_Qualifying Run: PBMC processing from whole blood. Graph indicates PBMC yields from 7 fresh whole blood samples from healthy donors with an average yield of 1.57 × 10^6 cells/mL and average viability of >90%. All cryopreserved samples were sent for reanalysis. There were 5/7 samples (S3-S7) that were thawed and analyzed at the UVRI/IAVI laboratory, Entebbe. Solid black circles indicate the PBMC yield on the day of the processing. Clear circles indicate recovery of stored samples in duplicate. Recovery of all 5 samples was >95%, well above the QA acceptance criteria of 60%.

(**Figure 1**). Mean recoveries were >94% for all samples and viabilities >90% (determined by manual counting and automated counting). Recovery data were also comparable between operators at the UVRI-IAVI HIV Vaccine Program laboratory, and no contamination of cultured cells was documented.

II. Qualifying-run Experiments

The purpose of the qualifying-run experiments is to ensure that all PMBCs isolated, frozen, thawed, and shipped from the laboratory meet the QA requirements and to obtain IAVI accreditation. The PBMC is isolated on site and witnessed by a staff member from the IAVI core lab, cryopreserved PBMCs, and analyzed for cell recoveries and viabilities. Aliquots of cryopreserved cells are sent to IAVI core lab for use in an enzyme-linked immunosorbent spot (ELISPOT) assay.

The QA criteria for the qualifying run are as follows:

- Expected cell yield from fresh blood on normal adult subjects or subjects on vaccine testing is between 0.8 × 10^6 and 3.0 × 10^6 cells/mL of blood.
- The acceptance criteria for frozen cell recovery and viability are: at Day 1: % recovery >70% and % viability >80%, and at Day 2: % recovery >60% and % viability >70%.
- The acceptable cell recovery from frozen cells for ELISPOT is >60% and viability of >70%.

Image 3_Masaka's Kiyumba Health Care Clinic.

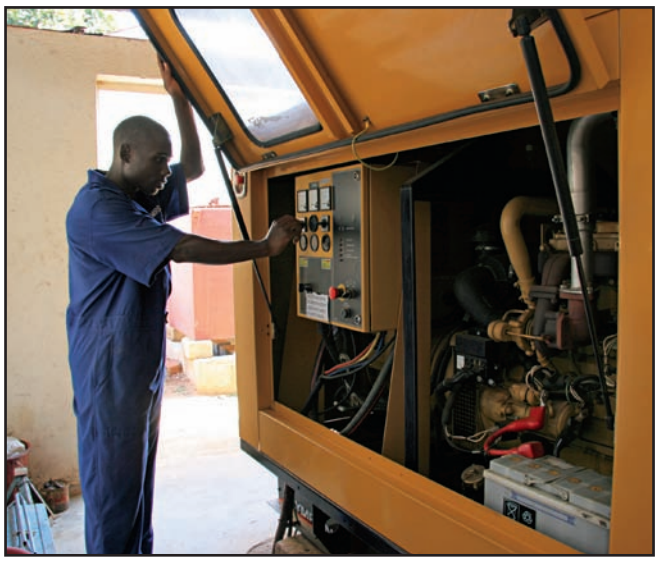

Image 4_Masaka site electrical generator.

Over a period of 1 week, PBMCs were isolated from fresh whole blood from 7 healthy donors, manually counted, and cryopreserved. A mean PBMC yield of 1.57 × 10^6 cells/mL and >98% viability was observed (**Figure 2**). Replicate cryopreserved vials were then sent to the UVRI/IAVI laboratory in Entebbe, where they were thawed, rested overnight, and analyzed by 2 independent operators. Of the 7 samples, 5 were chosen at random and analyzed; mean recoveries of >96% were observed and viabilities were >80%. No contamination of cells was documented. To further analyze the quality of cells obtained, ELISPOT assays were performed on the cryopreserved cells shipped to the IAVI core lab in London. Overall results and data quality was sufficient for the laboratory to pass the qualifying run (**Figure 3**).

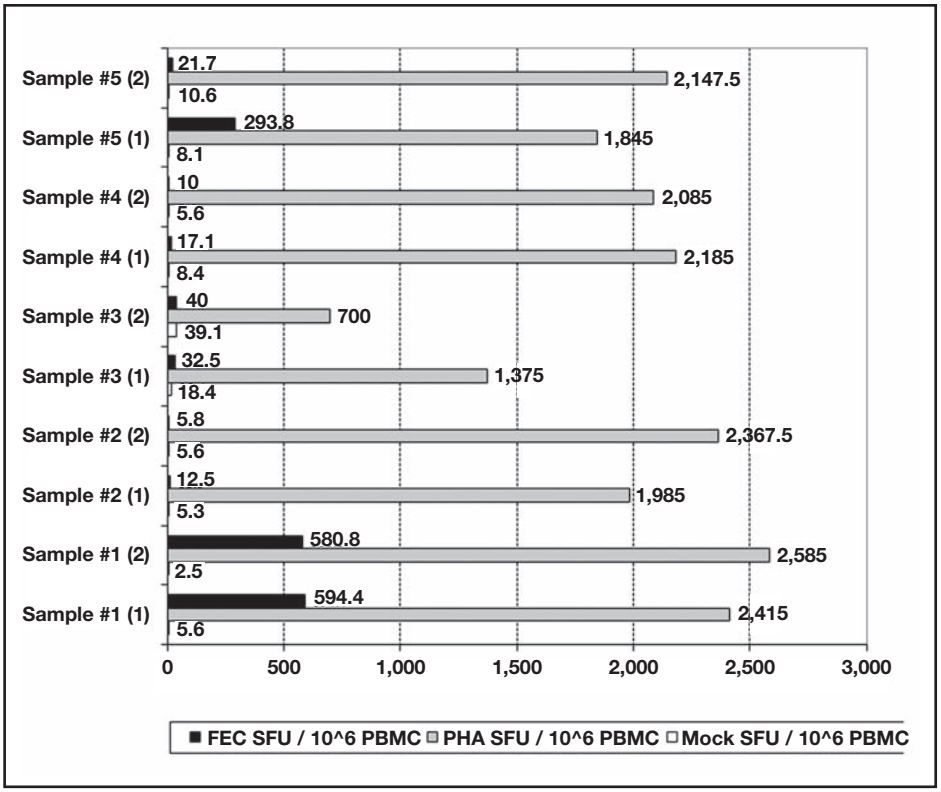

Figure 3_ELISPOT assay using cryopreserved PBMC cells from the Qualifying Run. ELISPOT was performed on 5 samples (Sample No. 1 - Sample No. 5) using the cryopreserved PBMCs. Two operators (designated [1] and [2]) performed ELISPOTS on the samples. One of the donors gave a discordant FEC response; Sample No. 3 (2) had high MOCK background (39.1 vs 18.4) as well as a low PHA response (700 vs 1375) compared to the duplicate; Sample No. 3 (1).

III. PBMC Yields Using 2 SOPs

The laboratory currently uses 2 different study protocols: the IAVI Acute HIV Infection Study and the SPARTAC trial protocols. The SOPs differ in the following key steps: 1) blood volume, 2) amount of histopaque per mL of blood, 3) speed and time of centrifugation steps, 4) reagents, 5) cell re-suspension volume, and 6) total processing time (**Table 2**). Using the IAVI-SOP, 12 healthy donors showed an average cell yield of 1.45 ± 0.34 × 10^6 cells/mL (**Figure 4**: group A). In 25 patients enrolled in the HIV acute infection study, the average yield was 1.28 ± 0.38 × 10^6 cells/mL samples (**Figure 4**: Group B). Using the SPARTAC SOP, 36 HIV early infected patients enrolled in the study showed an average cell yield of 1.01 ± 0.56 × 10^6 cells/mL (**Figure 4**: Group C). On average, PBMC processing using the IAVI-SOP took 4.06 ± 0.58 hours to

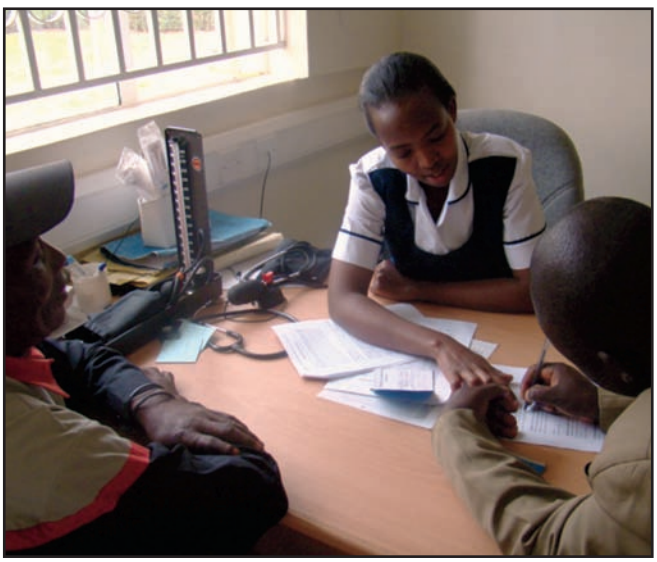

Image 5_Masaka staff obtaining patient consent.

Image 6_Masaka Cell Lab (MCL) technicians isolating Peripheral Blood Mononuclear Cells (PBMCs).

Table 1_PBMC Yield Using 2 SOPs

Group	Blood Vol. (mL)	Time (h)	Cell Yield (× 10^6 cells/mL)
A	64.62 ± 21.86	N/A	1.45 ± 0.35
B	85.02 ± 16.06	4.06 ± 0.58	1.28 ± 0.38
C	34.18 ± 1.22	3.18 ± 0.90	1.01 ± 0.56

Group A consists of 12 samples from healthy donors. Group B consists of 25 HIV+ samples from patients enrolled in the IAVI Protocol C study. Group C consists of 36 HIV+ samples from patients enrolled in the study. In group A and B, the IAVI-SOP was used, whereas in group C, the SPARTAC-SOP was used. Using patient samples, on average IAVI-SOP (Group B) took an average of 4.06 ± 0.58 per hour to process 85 ± 16 mL of blood with a PBMC yield of 1.28 ± 0.38 × 10^6 cells/mL. Using the SPARTAC-SOP (Group C) required an average of 3.18 ± 0.9 per hour to process 85 ± 34 ± 1.2 mL of blood with a PBMC yield of 1.01 ± 0.56 × 10^6 cells/mL.

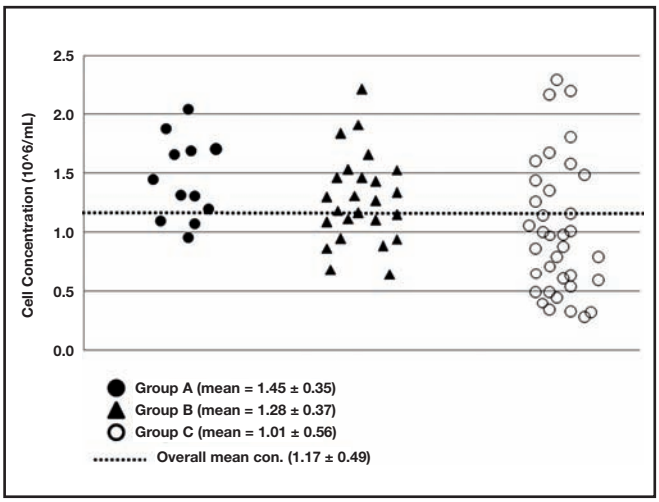

Figure 4_PBMC yield in 3 groups. Group A: PBMC yield in 12 healthy donors using the IAVI-SOP, with an average yield of 1.45 ± 0.35 × 10^6 cells/mL. Group B shows PBMC yields in 25 HIV+ samples from patients enrolled in the IAVI Protocol C study, with an average yield of 1.28 ± 0.37 × 10^6 cells/mL samples. Group C shows PBMC yields using SPARTAC-SOP, in 36 HIV+ samples from patients enrolled in the study with an average cell yield of 1.01 ± 0.56 × 10^6 cells/mL.

process 85 ± 16 mL of blood, whereas processing using the SPARTAC-SOP required an average of 3.18 ± 0.9 hours to process 34 ± 1.2 mL of blood (**Table 1**). A Bartlett's test was performed on cell yield per group, and a *P* value of 0.05 was obtained, indicating the variances of the measurement are not the same for the different groups. When we analyzed the cell yield of the HIV+ participants and adjusted for total blood volume and time of processing, it showed that blood volume (P=0.353, r^2=0.083) and total time of processing (P=0.900, r^2=0.083) did not have a significant correlation with cell yield.

IV. Comprehensive Audit Program

To maintain GCLP compliance, we implemented an audit program including: 1) internal audits, 2) bi-annual GCLP compliance audits by Clinical Support laboratory (CLS), Johannesburg, South Africa, 3) annual technical audits by IAVI, and 4) yearly PPD audits on all Division of AIDS (DAIDS)-funded studies.[19] Audits allow sponsors to validate laboratory systems prior to study initiation; they help identify deviations from policies and reinforce GCLP compliance. Following an audit, a report is issued detailing findings and recommendations, and, in response, the lab implements corrective actions to address findings. Two new SOPs were also developed and implemented after the audit; for example, an SOP for material rejection and acceptance criteria and an SOP for transport of samples clearly define how samples should be transported from the clinic to the lab for testing. The auditor also noted conflicting job titles in the organizational chart for the lab scientist, and this was addressed by updating the staff member's job description document. In another finding, the auditor noted that the thermometer used for the refrigerator/freezer temperature monitoring was not checked for accuracy, the point of action was ordering new certified thermometers for the refrigerator/freezer.

Discussion

The objective of setting up a standardized PBMC laboratory in our rural field site was to build local laboratory capacity in preparation and implementation of HIV vaccine

Image 7_Masaka Cell Lab (MCL) team working with the LIMS database.

Image 8_Masaka Clinic - internal data regulation.

Table 2_Difference Between the 2 SOPs

	IAVI SOP	SPARTAC SOP
Blood	Undiluted whole blood	Diluted whole blood
Vol. of histopaque	20 mL	25 mL
1st wash medium	Hank's balance salt solution without Ca+ or Mg+ (HBSS)	RPMI-PSG
1st centrifuge	400 g for 40 min at room temperature without breaks	800 g (220 rpm) for 20 min without breaks
2nd wash medium	Complete RPMI medium with 10% fetal calf serum	RPMI+PSG
2nd centrifuge	500 g for 10 min with breaks	Centrifuge at 536 g (1800 rpm) for 10 min with breaks
3rd centrifuge	400 g for 10 min with breaks	372 g (1500 rpm) for 8 min with breaks
Cell suspension volume for counting	5 mL	20 mL
Total time (min)	Average 246 min	Average 198 min

Table highlights the difference between the 2 SOPs. Difference is in the following key steps: 1) blood volume, 2) amount of histopaque per mL of blood, 3) speed and time of centrifugation steps, 4) reagents, 5) cell re-suspension volume, and 6) total processing time.

safety and efficacy trials and other immunological studies. We wanted to establish this capacity as close to the study population as possible, in order to decrease the total processing time of the samples, reduce transport costs, and produce consistent quality PBMC cell preparations. We successfully implemented this work according to GCLP principles.

The laboratory benefits from the IAVI Quality Program, which ensures GCLP is a continuous process. Good quality control on reagents, equipment, and general maintenance is performed. A policy is in place ensuring a regular supply of liquid nitrogen to the lab and a back-up stock of liquid nitrogen to last 10 days in case of supply disruption. A disaster recovery system is also in place involving a back-up laboratory. In our case, 2 laboratories in Entebbe can be used for this purpose: the lab of the Basic Science Programme of the MRC/UVRI Research Unit on AIDS and that of the UVRI-IAVI HIV Vaccine Program.

From the initial dry-run experiments performed at the newly established Masaka Laboratory, comparable PBMC yields were obtained by 2 operators on 2 blood samples. Recovery data from the 2 PBMC samples cryopreserved at the Masaka laboratory were within normal ranges when tested at the UVRI/IAVI laboratory, and no contamination of cultured cells was documented.

A qualifying run performed under the scrutiny of an IAVI trainer on a larger number of blood samples (n=7) processed and cryopreserved by 2 operators at the Masaka laboratory, and shipped to the UVRI/IAVI laboratory, yielded normal recovery and function by ELISPOT in 70%-80% of the samples analyzed, which is within the IAVI acceptance criteria. These results provided the basis for accreditation of the Masaka laboratory by IAVI.

In addition, larger numbers (n=12-36) of PBMC were processed by the Masaka laboratory using 2 different SOPs. The results demonstrated that cell yields were comparable between procedures and were not influenced by initial blood volume or the total time used for cell processing.

The use of the cryopreserved PBMCs in ELISPOT assays showed the PBMCs produced were of optimal quality and could be used in immunology assays to study HIV T cell responses in HIV vaccine-related studies. The laboratory had the capability to run 2 protocols with different PBMC processing SOPs. In both SOPs, PBMC yields were within the expected range (1.0-2.0 × 10^6 cells/mL) even though there were key differences in the 2 SOPs.

Audits, even though expensive and time consuming, were implemented to reassure staff, sponsors, and collaborators of the standards of the laboratory. The GCLP also allows the laboratory to implement effective site-specific policies. The laboratory received full GLCP accreditation from Qualogy.

Conclusion

We demonstrated feasibility of establishing a well-functioning PBMC processing laboratory in a remote field site in Africa. Currently, the laboratory has a limited capacity (1-4 patient samples/day), but it has the potential to expand and double this capacity if and when an extra biosafety cabinet and refrigerated centrifuge is acquired. Our experience could be adopted by other research institutions in developing countries that plan to perform HIV vaccine immunology-related work in remote sites. LM

Acknowledgments: We thank Prof. Heiner Grosskurth for his continuous support during the writing of this paper. Mr. Aloysius Ssemaganda and Mr. Paul Kato were invaluable in guiding us during the experiments and audits, and we express our sincere gratitude. We thank Dr. Jonathan Levin for his assistance in statistical analysis and the presentation of the data. We thank Mr. Tim Stiles for his continued guidance and for corrections made to this paper. Last, but not least, we extend our gratitude to all of the IAVI and SPARTAC teams for their support and help. This work was supported by the MRC (U.K.), by the Wellcome Trust through its funds provided to the SPARTAC Trial, and by the IAVI. We are indebted to the blood donors and study trial participants. LM

1. Nicholson JK, Green TA. Selection of anticoagulants for lymphocyte immunophenotyping. Effect of specimen age on results. *J Immunol Methods*. 1993;165:31-35.
2. Shalekoff S, Page-Shipp L, Tiemessen CT. Effects of anticoagulants and temperature on expression of activation markers CD11b and HLA-DR on human leukocytes. *Clin Diagn Lab Immunol*. 1998;5:695-702.
3. Kumar P, Satchidanandam V. Ethyleneglycol-bis-(beta-aminoethylether) tetraacetate as a blood anticoagulant: Preservation of antigen-presenting cell function and antigen-specific proliferative response of peripheral blood mononuclear cells from stored blood. *Clin Diagn Lab Immunol*. 2000;7:578-583.
4. Nicholson JK, Jones BM, Cross GD, et al. Comparison of T and B cell analyses on fresh and aged blood. *J Immunol Methods*. 1984;73:29-40.
5. Betensky RA, Connick E, Devers J, et al. Shipment impairs lymphocyte proliferative responses to microbial antigens. *Clin Diagn Lab Immunol*. 2000;7:759-763.

6. Bull M, Lee D, Stucky J, et al. Defining blood processing parameters for optimal detection of cryopreserved antigen-specific responses for HIV vaccine trials. *J Immunol Methods*. 2007;322:57-69.
7. Costantini A, Mancini S, Giuliodoro S, et al. Effects of cryopreservation on lymphocyte immunophenotype and function. *J Immunol Methods*. 2003;278:145-155.
8. Gilmour JW, Stevens WS, Gray C, et al. Laboratory expansion to large-scale international HIV preventive vaccine trials. *Curr Opin HIV AIDS*. 2007;2:201-206.
9. Yao K, McKinney B, Murphy A, et al. Improving quality management systems of laboratories in developing countries: An innovative training approach to accelerate laboratory accreditation. *Am J Clin Pathol*. 2010;134:401-409.
10. Zeh CE, Inzaule SC, Magero VO, et al. Field experience in implementing ISO 15189 in Kisumu, Kenya. *Am J Clin Pathol*. 2010;134:410-418.
11. Olmsted SS, Moore M, Meili RC, et al. Strengthening laboratory systems in resource-limited settings. *Am J Clin Pathol*. 2010;134:374-380.
12. Nkengasong JN. A shifting paradigm in strengthening laboratory health systems for global health: Acting now, acting collectively, but acting differently. *Am J Clin Pathol*. 2010;134:359-360.
13. Nkengasong JN, Nsubuga P, Nwanyanwu O, et al. Laboratory systems and services are critical in global health: Time to end the neglect? *Am J Clin Pathol*. 2010;134:368-373.
14. Gershy-Damet GM, Rotz P, Cross D, et al. The World Health Organization African region laboratory accreditation process: Improving the quality of laboratory systems in the African region. *Am J Clin Pathol*. 2010;134:393-400.
15. Sarzotti-Kelsoe M, Cox J, Cleland N, et al. Evaluation and recommendations on good clinical laboratory practice guidelines for phase I-III clinical trials. *PLoS Med*. 2009;6:e1000067.
16. Stiles T, Grant V, Mawloy N, et al. Good Clinical Laboratory Practice (GCLP)—A quality system for laboratories that undertake the analyses of samples from clinical trials, 2003.
17. Ezzelle J, Rodriguez-Chavez IR, Darden JM, et al. Guidelines on good clinical laboratory practice: Bridging operations between research and clinical research laboratories. *J Pharm Biomed Anal.* 2008;46:18-29.
18. Crucitti T, Fransen K, Maharaj R, et al. Obtaining valid laboratory data in clinical trials conducted in resource diverse settings: Lessons learned from a microbicide phase III clinical trial. *PLoS One*. 2010;5:e13592.
19. DAIDS Guidelines for Good Clinical Laboratory Practice Standards-Training. Available at: www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/gclp.pdf. Accessed November 25, 2010.