Transactions of the Royal Society of Tropical Medicine and Hygiene (2006) **100**, 980—985

available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevierhealth.com/journals/trst

The Partec CyFlow Counter® could provide an option for CD4+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi

M. Fryland[a], P. Chaillet[b], R. Zachariah[c,*], A. Barnaba[d], L. Bonte[e], R. Andereassen[a], S. Charrondière[f], R. Teck[a], O. Didakus[f]

[a] *Médecins sans Frontières—Luxembourg, Thyolo District, Malawi*
[b] *Médecins sans Frontières, Medical Department, Brussels Operational Center, Belgium*
[c] *Médecins sans Frontières, Operational Research (HIV—TB), Medical Department, Brussels Operational Center, Brussels, Belgium*
[d] *Reference Laboratory Unit, Ministry of Health and Population, Lilongwe, Malawi*
[e] *Médecins sans Frontières—France, Medical Department, Paris, France*
[f] *Médecins sans Frontières—France, Chiradzulu District, Malawi*

Received 5 September 2005; received in revised form 29 November 2005; accepted 29 November 2005
Available online 20 March 2006

KEYWORDS
HIV;
CD4;
Antiretroviral therapy;
ART;
CyFlow Counter®;
Malawi

Summary A study was conducted in rural Malawi to verify (a) whether the Partec CyFlow Counter® for CD4+ T-cell lymphocyte counting in HIV-positive individuals could be introduced into a district hospital laboratory and (b) whether it would produce CD4 counts of acceptable quality. CD4+ cell counting was performed using the Partec CyFlow Counter and the results were compared with a reference method (FACsCount). A total of 311 blood samples were analysed and the correlation coefficient for the CyFlow Counter was 0.92 (95% CI 0.89—0.95). Mean CD4 counts using the Partec and the reference methods were 308.2 cells/μl and 316.9 cells/μl, respectively. The mean difference in CD4 count values was −8.68 cells/μl (95% CI −18.8 to 1.4). Mean intra-run variation was −6.84 cells/μl (95% CI −12.9 to 0.79). In the district laboratory setting, the instrument could accommodate up to 75 blood samples per technician per day. After being trained, local laboratory staff found the CyFlow Counter procedures simple to run and the instrument easy to manipulate. The Partec CyFlow Counter produces sufficiently reliable results and the instrument appears robust under field conditions. It could provide a new option for introducing routine CD4+ cell monitoring at the district level in the context of scaling-up antiretroviral therapy in Malawi.

* Corresponding author. Present address: Médecins sans Frontières, Brussels Operational Center, Operational Research (HIV—TB), Medical Department, 68 Rue de Gasperich, L-1617, Luxembourg. Tel.: +352 332 515; fax: +352 335 133.
E-mail address: zachariah@internet.lu (R. Zachariah).

0035-9203/$ — see front matter
doi:10.1016/j.trstmh.2005.11.014

1. Introduction

Malawi is a small and impoverished country in southern Africa with 10 million inhabitants. In 2003 there were an estimated 900 000 people living with HIV/AIDS and 86 000 AIDS-related deaths (MOHP, 2003a). In the same year, an estimated 170 000 people were considered to be in immediate need of antiretroviral therapy (ART). Malawi has thus laid out an ambitious plan to scale-up ART to 80 000 people by the end of 2005 (MOHP, 2003b).

CD4+ T-lymphocyte counting is an important parameter for monitoring individuals with HIV/AIDS as CD4+ cell counts are the best indicator for (a) assessing the risk of occurrence of opportunistic infections, (b) deciding when to initiate ART and (c) monitoring immunological response to therapy (Saravolatz et al., 1996). Despite its importance, available CD4 technology remains for the large part expensive and inappropriate for ART scale-up in developing countries.

Flow cytometry is considered the gold standard technique for CD4+ cell enumeration (CDC, 1997; Landay et al., 1990). Nevertheless, traditional flow cytometry instruments such as FACsScan and FACsCalibur (Becton Dickinson) have several limitations. The equipment and reagents are exorbitantly expensive, specialised infrastructure is required for their installation, and skilled manpower is needed for routine use and maintenance (Diagbouga et al., 2003; Greve et al., 2003; Janossy et al., 2000; Mandy et al., 2002; Sherman et al., 1999).

The FACsCount (Becton Dickinson) is a simplified flow cytometer (Lopez et al., 1999; Young et al., 1997) but is still very expensive. Several manual techniques that overcome the problem of sophistication exist (Didier et al., 2001; Lyamuya et al., 1996). However, they are limited by a low throughput of samples and high interpersonal variation in results as well as being labour intensive. The latter is of particular relevance to the situation in Malawi where there is a serious lack of human resources and where laboratory staff are already overworked (Kober and Van Damme, 2004; MOHP, 2003c).

To avoid universal CD4 monitoring acting as a barrier to the national scale-up strategy in Malawi, the country currently offers ART to all individuals who are assessed clinically as being in WHO stages III or IV and treatment response is monitored clinically (WHO, 2003). Since an unavoidable delay exists between immunological and clinical failure, such a strategy thus carries a risk that treatment failure is detected late and might be associated with the development of drug resistance. The overall effectiveness of ART in promoting survival where the HIV burden is highest may thus be compromised. The need for practical, field-friendly and cheap methods of measuring CD4+ lymphocyte counts in the context of scaling-up ART is thus urgent (Mwaba et al., 2003).

Recently, an instrument called the Partec CyFlow Counter® (Partec GmbH, Münster, Germany) has become available for CD4+ T-cell enumeration (www.cytecs.com). The instrument is relatively cheap and the cost of reagents is 5—20 times cheaper than that for FACsCount or other available methods. It does not depend on a stable power supply, can work on a car battery and can be moved between sites. It is also relatively small, reputed to be easy to use and has a high throughput of samples.

The CyFlow Counter may thus provide an alternative technique for CD4 monitoring in the context of the imminent scaling-up of ART in Malawi. However, this instrument has not been evaluated in resource-limited settings and we are unsure whether its claimed merits are reproducible in this setting.

We thus decided to assess (a) whether the Partec CyFlow Counter could be introduced into a routine rural district laboratory in Malawi and (b) whether it would produce CD4 results of acceptable quality.

2. Methods

2.1. Study setting and population

The study was conducted between August and November 2003 in Thyolo and Chiradzulu districts in rural southern Malawi. The two districts have populations of approximately 450 000 and 160 000 inhabitants, respectively, and are the pioneers to pilot HIV/AIDS-related activities, including ART, in Malawi. Since early 1999, the two hospitals offer voluntary counselling and HIV testing (VCT) to ill patients, mothers attending antenatal care, patients with tuberculosis and all those who wish to know their HIV status. ART is offered according to national guidelines and is free of charge (MOHP, 2003c). All consecutive HIV-positive individuals in various WHO stages of disease presenting either to the VCT unit or to the HIV/AIDS clinics were included in the study. Thirty-five consecutive HIV-negative individuals who presented to VCT were also included to assess instrument accuracy at CD4 counts that are likely to be higher than in HIV-positive individuals. This study received ethical approval from the National Health Sciences Research Council of Malawi and the Ethical Review Board of Médecins sans Frontières.

2.2. The CyFlow Counter, and intra-run and inter-run variation

The CyFlow Counter is a fully equipped, portable, ultra-compact desktop flow cytometer dedicated principally to CD4 counting (but is also for CD8 and CD3 counting). The instrument uses fluorescent-labelled anti-CD4 monoclonal antibodies to capture CD4+ T-cells from whole blood and allows automated CD4 counting. The CyFlow Counter was installed at the Chiradzulu district hospital laboratory by a local laboratory team; technical support was provided by the manufacturer. No specific changes to the infrastructure were made to cater for installation of this instrument.

Intra-run variation was assessed in two different ways. Ten consecutive CD4 readings were performed on the same single blood preparation in the same tube. The second procedure involved repeating the CD4 count reading on 84 different samples. Inter-run variation was assessed by comparing CD4 counts from ten runs carried out on the same blood sample prepared in ten separate tubes. Inter-personal variation was assessed by comparing results obtained by three different laboratory technicians.

The Chiradzulu laboratory is a typical district hospital laboratory in Malawi that runs a standard essential package of laboratory tests according to national guidelines (MOHP,

2003c). Laboratory technicians were trained in procedures related to blood preparation and operating the instrument.

2.3. Blood collection and CD4 counting

After obtaining informed consent, two aliquots of blood were collected by sterile venipuncture into tubes containing EDTA anticoagulant. CD4+ T-cell enumeration was performed using the CyFlow Counter and a parallel analysis was performed using an accepted reference instrument, the FACsCount, in an independent reference laboratory. The results from the FACsCount were designated as the gold standard for this study.

The CyFlow Counter uses a 'no lyse, no wash' procedure for CD4 counting (Greve et al., 2003). Fifty microlitres of EDTA-anticoagulated blood were added to 10 μl of monoclonal antibodies. After 15 min of incubation, 1 ml of no lyse dilution buffer was added and the sample tube was attached to the CyFlow Counter for automated counting. Results were available in 2 min and were expressed in a histogram (CD4+ cells/μl).

CD4 counting using the FACsCount similarly involved adding 50 μl of EDTA-anticoagulated blood to ready-to-use tubes. After incubation for 60 min, 50 μl of fixative solution was added. This was followed by a second incubation period of 30 min, following which samples were ready for acquisition. CD4 count results were available in 3—7 min. All blood samples were processed on the same day as blood draw. A vehicle was made available for transport of blood samples from Thyolo district to the Chiradzulu district hospital laboratory as well as to the reference laboratory located in Blantyre, Malawi. Partec PreCount Control beads and FACsCount controls were used for internal quality control of CD4 test results for the two respective instruments.

2.4. Statistical analysis

Data acquisition and analysis were performed in real time with a standard Pentium PC. Analysis was performed using Method Validator Version 1.1.9.0.

The absolute CD4 count was the variable of interest. Intra- and inter-run variability were assessed using the mean value. CD4 count results from the CyFlow Counter were compared with those derived from FACsCount (the gold standard) using a Bland—Altman method of analysis (Bland and Altman, 1986). The measure of linear association was expressed as a correlation coefficient. The mean difference in CD4 counts by the two methods was also determined. The confidence interval (CI) was set at 95% with a 5% error risk.

3. Results

3.1. Characteristics of the study population

A total of 318 individuals were registered for the study. Seven individuals were excluded; five blood samples introduced for counting by FACsCount did not produce results, one sample was not drawn in aliquot and one sample was clotted. Of the 311 individuals who were included in the study, there were 109 (35%) men and 202 (65%) women, with a median age of 34 years (range 2—80 years).

Two hundred and seventy-six individuals were HIV-positive and 35 were HIV-negative. Of the HIV-positive individuals, 19 (7%) were classified in WHO stage I, 90 (33%) were in WHO stage II, 121 (44%) were in WHO stage III and 46 (17%) in WHO stage IV. The median CD4 count in all blood samples was 212 cells/μl (range 2—1789 cells/μl).

3.2. Intra-run and inter-run variation

Intra-run variation on the ten consecutive CD4 readings performed on the same single blood preparation in the same tube was 3%. The mean intra-run variation was −6.84 cells/μl (95% CI − 12.9 to 0.79) on repeat reading of 84 different samples. Inter-run variation was 6%. Inter-personal variation in CD4 counts between three different laboratory technicians was 5% in high CD4+ T-cell counts above 800 cells/μl and 16% in CD4+ T-cell counts under 50 cells/μl.

3.3. CD4 counting using the CyFlow Counter and FACsCount

Figure 1 demonstrates the linear regression analysis between the CD4 count results obtained from the CyFlow Counter with that of the reference method (FACsCount). The correlation coefficient of the CyFlow Counter was 0.92 (95% CI 0.89—0.95). The mean CD4 count using the Partec instrument was 308.2 cells/μl whilst that with the reference method was 316.9 cells/μl. The mean difference in CD4 count values between the two methods was −8.68 cells/μl (95% CI −18.8 to 1.4) (Figure 2).

The throughput of the CyFlow Counter was estimated at 75 samples per technician per day when used in a district

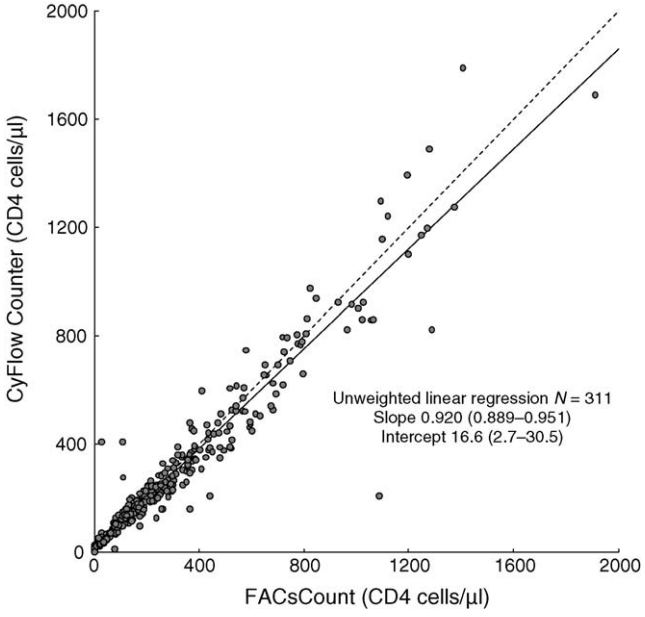

Figure 1 Regression analysis between the CyFlow Counter and FACsCount with the theoretical (dotted line) and real (full line) coefficient.

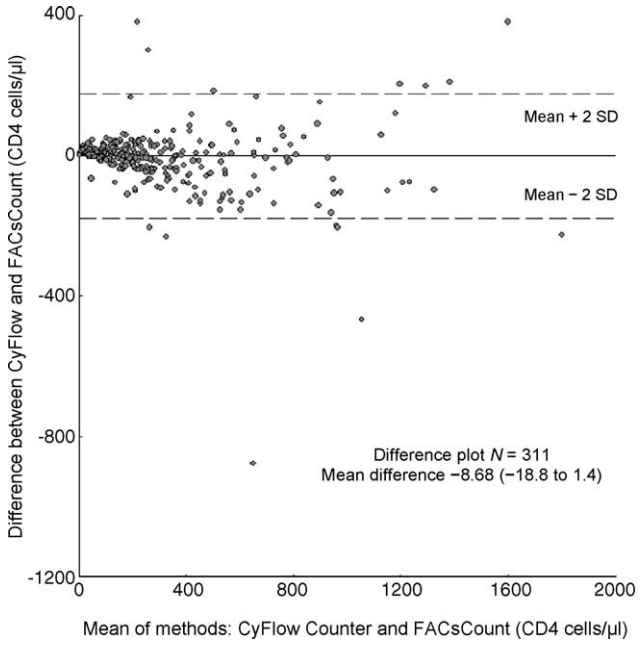

Figure 2 Mean difference (CD4+ cells/μl) between the CyFlow Counter and FACsCount.

hospital setting and including systematic daily internal quality control runs.

4. Discussion

This study shows that it is feasible to install and run the Partec CyFlow Counter in a routine district level laboratory in Malawi and the CD4+ T-cell counts are of acceptable quality.

The results produced by this machine in a district hospital laboratory showed a correlation of 92% with a very limited mean difference compared with the reference method. The CD4 count is the product of three variables — the white blood cell count, percent lymphocytes and the percent CD4+ T-cells — and analytical variations (even with the same instrument) are normal. This accounts for the 'wide range' within normal CD4 values (usually between 500 and 1400 cells/μl). From a therapeutic decision point of view, the results from the Partec CyFlow Counter are thus very satisfactory. This becomes all the more acceptable as it means that CD4 counting could be provided at the local level. Two other studies from different settings have similarly shown that accurate determinations of CD4+ T-lymphocytes can be obtained with the Partec CyFlow Counter (Cassens et al., 2004; Dieye et al., 2005.).

Our experience with this instrument is encouraging for a number of other operational reasons. First, the instrument is small, portable and could be installed in a rural district laboratory equipped with very basic infrastructure and amenities (a running water supply and electricity supply on the district grid). The local technicians quickly became conversant with the counting procedures and use of the machine.

Second, sample preparation for CD4 counting with the CyFlow Counter took approximately 15 min compared with 90 min for the FACsCount. In a setting where laboratory technicians are few, have limited time and are overworked (Kober and Van Damme, 2004; MOHP, 2003d), the CyFlow Counter is thus 'technician friendly'.

Newer generation CyFlow Counters now benefit from an alignFree™ optical technology that spares the problem of difficult and often time consuming optical alignment and adjustment. This reduces the need for regular technical support and maintenance. In a resource-limited setting such as Malawi, this is an important operational consideration. An important limitation of this study is the fact that the evaluation covered a relatively short period of time and is thus unable to provide information on how robust the CyFlow Counter will be on a longer-term basis.

There are a number of operational perspectives that tend to favour the use of the CyFlow Counter for CD4 monitoring in Malawi. First, the current scaling-up plan for Malawi is focused on districts on a country-wide level (MOHP, 2003b). A district such as Thyolo, with approximately 450 000 inhabitants, has an estimated 40 500 people living with HIV/AIDS (global HIV prevalence of 9%) of whom approximately 7000 are thought to require ART. The current target is to place half of this number (3500) on ART by the end of 2005. If three CD4 counts were to be performed per person per year (at baseline, 6 and 12 months), there will be a need for the district laboratory to perform approximately 10 500 CD4 tests per year. With a throughput of 75 tests per technician per day and assuming 20 working days per month, our laboratory could run 18 000 CD4 tests per year. The CyFlow Counter would thus have adequate capacity to cope with the CD4 testing needs linked to the target of placing 3500 people on ART by the end of 2005.

Second, ART initiation and follow-up will progressively have to be decentralised to more distant peripheral health facilities that are unlikely to have basic laboratory facilities. The Partec CyFlow Counter is portable and can apparently be operated within a vehicle on a car battery (www.cytecs.com). Although we have not tested the feasibility of this approach, the machine opens the potential of making CD4 monitoring available on a 'mobile basis' at peripheral health facilities. Such a strategy might allow access to 'same day' CD4 count results for individuals who are being managed at distant health facilities and are who unable to make the distance to the district hospital for whatever reason. Where both financial and human resource capacity is limited, such as in Malawi, a mobile approach is likely to be associated with considerable cost advantages and will optimise 'capacity sharing' within a network of health facilities.

The other main operational consideration is the cost of reagents, for which the CyFlow Counter offers a considerable advantage compared with the FACsCount (Becton Dickinson) or manual techniques such as Dynabeads® technology (Dynal Biotech, Oslo, Norway) (Diagbouga et al., 2003). With the same above example of an estimated 10 500 CD4 tests required per year for Thyolo district, the cost (at the time of study) in terms of CyFlow reagents would be US$21 000 (US$2/test), whilst that for FACsCount would be US$147 000 (US$14/test) and US$52 500 (US$5/test) for Dynabeads. The investment cost of the CyFlow Counter and FACsCount instruments, which have a high enough throughput to be used in district hospitals, amounts to more or less US$20 000. Making such an investment for each of the

50 district hospitals country-wide in Malawi would mean US$1 million for instruments alone. This is a considerable investment compared with currently available resources for ART in high prevalence settings such as Malawi!

The desired evolution of this much needed technology should be one that has been reached for rapid whole blood testing for HIV (WHO, 2004) using techniques such as rapid slide agglutination or dipstick-like tests that are affordable and simple to use. The obvious practical advantages would include: increased numbers of people who will benefit from CD4 count monitoring; test results can be obtained quickly (on the same day); less reliance will need to be placed on laboratory services or sophisticated equipment; and CD4 counting could be made available at decentralised sites such as health centres.

The international community recognises the pressing medical, moral, social and economic imperatives to expand access to ART to the many people living with HIV/AIDS in the developing world. Countries such as Malawi have taken up this challenge of offering ART to the thousands in need. Despite the fact that the CD4 count remains an undeniable part of what is considered 'optimal patient management', countries such as Malawi are currently unable to offer universal CD4 count monitoring. It is now beholden on manufacturing companies and research institutions to take up the challenge of simplifying CD4 technology and making it much more affordable and accessible for the thousands of HIV-positive individuals in some of the most impoverished parts of the world that urgently need access to this technology.

Conflicts of interest statement
The authors have no conflicts of interest concerning the work reported in this paper.

Acknowledgements

We are grateful to the Thyolo and Chiradzulu district management teams and to the National AIDS Commission, Ministry of Health and Population of Malawi for collaboration and encouragement in trying to implement HIV/AIDS-related activities including ART in these two districts. We are grateful to the district laboratory technicians in Thyolo and Chiradzulu (particularly Winfred) as well as the Médecins sans Frontières (MSF) teams in both districts for their support for this study. We thank Dr Pierre Humblet and Dr Peter Firmenich for their useful comments on the protocol and the manuscript. We are particularly grateful to the Welcome Trust Laboratory in Blantyre, Malawi, for conducting FACsCount testing for this study. This study was financed by MSF.

References

Bland, J.M., Altman, D.G., 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1, 307—310.

Cassens, U., Gohde, W., Kuling, G., Groning, A., Schlenke, P., Lehman, L.G., Traore, Y., Servais, J., Henin, Y., Reichelt, D., Greve, B., 2004. Simplified volumetric flow cytometry allows feasible and accurate determination of CD4 T lymphocytes in immunodeficient patients worldwide. Antivir. Ther. 9, 395—405.

CDC, 1997. 1997 revised guidelines for performing CD4+ T-cell determinations in persons infected with human immunodeficiency virus (HIV). Centers for Disease Control and Prevention. MMWR Recomm. Rep. 46, 1—29.

Diagbouga, S., Chazallon, C., Kazatchkine, M.D., Van de Perre, P., Inwoley, A., M'Boup, S., David, M.P., Tenin, A.T., Soudre, R., Aboulker, J.P., Weiss, L., 2003. Successful implementation of a low-cost method for enumerating CD4 T lymphocytes in resource-limited settings: the ANRS 12—26 study. AIDS 17, 2201—2208.

Didier, J.M., Kazatchkine, M.D., Demouchy, C., Moat, C., Diagbouga, S., Sepulveda, C., Di Lonardo, A.M., Weiss, L., 2001. Comparative assessment of five alternative methods for CD4+ T-lymphocyte enumeration for implementation in developing countries. J. Acquir. Immune Defic. Syndr. 26, 193—195.

Dieye, T.N., Vereecken, C., Diallo, A.A., Ondoa, P., Diaw, P.A., Camara, M., Karam, F., Mboup, S., Kestens, L., 2005. Absolute CD4 T-cell counting in resource-poor settings: direct volumetric measurements versus bead-based clinical flow cytometry instruments. J. Acquir. Immune Defic. Syndr. 39, 32—37.

Greve, B., Cassens, U., Westerberg, C., Ghde, W., Sibrowski, W., Reichelt, D., 2003. A new no-lyse, no wash flow-cytometric method for the determination of CD4 T cells in blood samples. Transfus. Med. Hemother. 30, 8—13.

Janossy, G., Jani, I., Gohde, W., 2000. Affordable CD4(+) T-cell counts on 'single-platform' flow cytometers I. Primary CD4 gating. Br. J. Haematol. 111, 1198—1208.

Kober, K., Van Damme, W., 2004. Scaling up access to antiretroviral treatment in southern Africa: who will do the job? Lancet 364, 103—107.

Landay, A., Ohlsson-Wilhelm, B., Giorgi, J.V., 1990. Application of flow cytometry to the study of HIV infection. AIDS 4, 479—497.

Lopez, A., Caragol, I., Candeias, J., Villamor, N., Echaniz, P., Ortuno, F., Sempere, A., Strauss, K., Orfao, A., 1999. Enumeration of CD4(+) T-cells in the peripheral blood of HIV-infected patients: an interlaboratory study of the FACSCount system. Cytometry 38, 231—237.

Lyamuya, E.F., Kagoma, C., Mbena, E.C., Urassa, W.K., Pallagyo, K., Mhalu, F.S., Biberfeld, G., 1996. Evaluation of the FACScount, TRAx CD4 and Dynabeads methods for CD4 lymphocyte determination. J. Immunol. Methods 195, 103—112.

Mandy, F., Nicholson, J., Autran, B., Janossy, G., 2002. T-cell subset counting and the fight against AIDS: reflections over a 20-year struggle. Cytometry 50, 39—45.

MOHP, 2003a. National AIDS Commission of Malawi. National estimate of HIV/AIDS in Malawi. Ministry of Health and Population, Lilongwe, Malawi.

MOHP, 2003b. Treatment of AIDS. The two year plan to scale up ART in Malawi (2004—2005). Ministry of Health and Population, Lilongwe, Malawi.

MOHP, 2003c. The status of human capacity development for an effective response to the HIV/AIDS epidemic in Malawi (evaluation report). Ministry of Health and Population, Lilongwe, Malawi.

MOHP, 2003d. Guidelines for the use of anti-retroviral therapy in Malawi, second ed. Ministry of Health and Population, Lilongwe, Malawi.

Mwaba, P., Cassol, S., Pilon, R., Chintu, C., Janes, M., Nunn, A., Zumla, A., 2003. Use of dried whole blood spots to measure CD4+ lymphocyte counts in HIV-1-infected patients. Lancet 362, 1459—1460.

Saravolatz, L., Neaton, J.D., Sacks, L., Deyton, L., Rhame, F., Sherer, R., 1996. CD4+ T lymphocyte counts and patterns of mortality among patients infected with human immunodeficiency virus who were enrolled in community programs for clinical research on AIDS. Clin. Infect. Dis. 22, 513—520.

Sherman, G.G., Galpin, J.S., Patel, J.M., Medelow, B.V., Glencross, D.K., 1999. $CD4^+$ T-cell enumeration in HIV infection with limited resources. J. Immunol. Methods 222, 209—217.

WHO, 2003. Scaling up anti-retroviral therapy in resource-limited settings. Treatment guidelines for a public health approach. World Health Organization, Geneva, WHO, QV268.5 (revised version).

WHO, 2004. Rapid HIV tests. Guidelines for use in HIV testing and counselling services in resource-constrained settings. World Health Organization, Geneva.

Young, N.L., Ponglertnapakorn, P., Shaffer, N., 1997. Clinical field site evaluation of the FACsCount for absolute $CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$ cell count determinations in Thailand. Clin. Diagn. Lab. Immunol. 4, 783—786.