

**THE IMPACT OF A CD4 EXTERNAL QUALITY ASSESSMENT PROGRAMME
FOR SOUTHERN AFRICA AND AFRICA**

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of
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DECLARATION

**I, Hazel Aggett declare that this dissertation is my own work.
It is being submitted for the degree of Master of Science in Medicine in the branch of
Molecular Medicine and Haematology in the University of Witwatersrand,
Johannesburg.**

**It has not been submitted before for any degree or examination at this or any other
University.**

.....

.....day of.....2009

DEDICATION

To my husband, Nicholas Aggett, for all his endless patience and support.

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1. *CD4 testing of HIV positive patients in South Africa is internationally comparable.* Lawrie D, Aggett HM, Tepper M, Grimett S, Marshall T, Cassim N and Glencross DK.
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Implementation of a CD4 REQAS. Implications for standardised PLG CD4 testing.
Aggett H and Glencross DK.

ABSTRACT

Background: The aim was to review the first twenty trials of the regional CD4 AFREQAS scheme. This scheme was implemented to raise awareness of the need for quality laboratory testing and to continually improve and maintain a high standard of CD4 enumeration used in support of treatment programmes. The overall performance from 2002 to 2006 (Trials 1-20) is reported.

Methods: Commercial stabilised CD4 external quality assessment (EQA) material, both with normal and low CD4 values were shipped across 20 trials. Data was analysed for each trial, for each participant which included; the trimmed mean, standard deviation, the percent coefficient of variation, the residual, and the standard deviation index (SDI) values for both the CD4 absolute counts (CD4 abs counts) and CD4 lymphocyte percentages (CD4%Ly). Individual laboratory SDI values across 20 trials were analysed according to CD4 methods. The cumulative pooled SDI data across 20 trials of subgroups of users, using similar methods were analysed. The overall performance of Southern African networks and Grant supported networks were assessed.

Results: Overall CD4 AFREQAS between-laboratory precision (trimmed %CV) was 11.9% and 10.8% for CD4 abs counts and CD4%Ly respectively. For the respective CD4 abs counts and CD4%Ly where normal value material was shipped, trimmed %CV of 10.9% and 8.1% were observed, and in low value shipments trimmed %CV of 14.2% and 17.0% were observed. The cumulative CD4 abs counts SDI analysis showed the best between-laboratory precision amongst BDS FACSCount™ and Panleucogating (PLG-CD4) users (both SD (SDI) =<1.2 and %CV of <9%). Single platform algorithm based systems (BDS TruCOUNT™ MultiSET™) and volumetric systems (Partec®) showed poorer between-laboratory precision (both SD (SDI) =>2 and %CV of >13%). These two systems had more outliers and no submission results than the BDS FACSCount™ and PLG-CD4 users. With respect to the African and Grant supported networks, the CD4 AFREQAS scheme has highlighted a number of factors that have contributed to poor performance which are lack of training and poor service delivery. Investment in additional technical training by the service providers and improved service delivery will improve the quality of CD4 testing in Africa.

Conclusion: From the inception of CD4 AFREQAS, significant advances have been made in the quality control and monitoring of participating national and African laboratories offering CD4 testing. CD4 AFREQAS has provided a platform for improving the reproducibility of CD4 reporting both within and between laboratories in South Africa and Africa. With ongoing teaching through participant feedback evaluation, and dedicated training programmes, as well facilitating the use of standardised laboratory protocols necessary to contain costs, and maintain high standards of CD4 testing, there has been a huge impact on establishing excellent CD4 service delivery.

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ABBREVIATIONS

%CV	Percent coefficient variation
ADR	Agreement of Dangerous goods by road and rail
AFREQAS	African Regional Quality Assessment Scheme
AFRO	AFRICAN (WHO)
AIDS	Acquired immune deficiency syndrome
ARV	Antiretroviral
CAP	College of American Pathologists
CD4 abs	CD4 absolute count
CD4%Ly	CD4 percentage of lymphocytes
CDC	Centre for Disease Control
CLIA	Clinical Laboratory Improvement Act
DP	Dual platform
ELISA	Enzyme linked immunosorbent assay
EQA	External Quality Assessment
FCR	Flow count rate
FDA	Food and Drug Administration
GCLP	Good Clinical Laboratory Practice
HIV	Human immunodeficiency virus
IATA	International Air Transport Association
IAVI	International Aids Vaccine Initiative
NHLS	National Health Laboratory Service
PCR	Polymerase chain reaction
PEPFAR	United States president emergency plan for AIDS relief
PT	Proficiency Testing
QASI	Quality Assurance Systems International
SD	Standard deviation
SDI	Standard deviation index
SP	Single platform
UK NEQAS	United Kingdom National Quality Assessment Service
WHO	World Health Organization

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1.0 INTRODUCTION

An external quality assessment (EQA) scheme is designed to provide an independent check of laboratory results. It allows comparison of different methods and instruments for the same test and therefore is an assessment of reliability between laboratories. External quality assessment (EQA) is an evaluation by an outside organization of the performance by a number of laboratories on specially supplied control samples. The EQA scheme monitors the performance of each laboratory over time and identifies those laboratories that require training or corrective action to improve their performance. One of the best ways for a laboratory to monitor its performance, against both its own requirements and the performance of other laboratories, is to participate regularly in an EQA scheme. This testing helps to highlight not only repeatability (same method) and reproducibility (different methods for same test) performance between laboratories, but also systematic errors, i.e. bias or trends.

Analysis of performance of a laboratory is however, a retrospective exercise and cannot override good internal quality control procedures. The EQA provides the laboratory with a snapshot of what would have occurred on a “normal” routine day in a laboratory. The objective of an EQA scheme is to promote between-laboratory and between-method comparability, whereas, it is the prerogative of a laboratory to attain good within laboratory precision. The EQA scheme enables participating laboratories to relate their performance to their peers, either nationally or internationally.

Good Clinical Laboratory Practice (GCLP) (1) enables maintaining quality. Good Clinical Laboratory Practice encompasses quality control comprising (i) Internal quality control: the laboratory uses procedures and reagents recommended by the manufacturers to ensure accuracy and reproducibility of the testing system, test by test, day by day and (ii) External quality assessment (EQA): an evaluation by an outside organization of the performance by a number of laboratories on specially supplied control samples. Quality control is supported by the procedures performed with a set of operating procedures to ensure standardization of testing.

1.1 Background of Current CD4 EQA Schemes

External quality assessment schemes in flow cytometry play an important role of laboratory testing of immune monitoring and testing. In the last twenty years, several EQA schemes were in operation in the United States, Canada and Europe with the purpose to improve the accuracy and precision of cell identification and testing of lymphocyte subsets and leukaemia/lymphoma immunophenotyping. With the outbreak of the acquired immune deficiency syndrome (AIDS) epidemic and the need for reliable CD4 testing, scientists and pathologists were given a stimulus to develop accurate and reliable methods for enumeration of CD4 cells (2). This led to an increased demand for EQA in flow cytometry and initiated a series of multinational collaborative studies (3-6). Laboratories can benefit from participation in an EQA scheme. It confirms the competency of the laboratory, identifies problems with test methods, monitors participant improvement of performance over time, educates staff, generates confidence, monitors competency of staff, determines accuracy and precision of test methods and can satisfy local auditing and accreditation organisations, depending on the type of scheme used by a laboratory (7).

There are two types of quality assessment schemes available (8, 9):

1. Proficiency testing (PT) refers to an external quality assessment scheme in which sanctions are linked to inadequate performance; for example, a laboratory may be required to repeat testing if poor performance is noted on a single trial. These laboratories can however have their accreditation status rescinded in the event of ongoing poor performance and they would only be re-instated once adequate performance is re-established. This type of assessment scheme is mandatory for all laboratories in the United States of America, where all PT schemes are required by law to subscribe to the guidelines by the Clinical Laboratory Improvement Act (CLIA) (10-12).
2. The other type of EQA scheme is an assessment scheme where there are no penalties for poor performance and the emphasis is on improving quality of testing through technical support. There are many debates about the differences between, and the benefits of educational and regulatory EQA schemes. An educational EQA scheme appeals to more participants than a regulatory PT scheme. On the downside, because educational EQA schemes are not compulsory or enforced by

law, the return of results may be lower compared to participants in regulatory schemes (13). The EQA schemes practiced in the Benelux countries and by UK NEQAS are recognized as the model best fulfilling participant's needs and as more effective than the ones run by the Federal Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) in the United States (14). The European and United Kingdom EQA schemes are not only regulatory bodies, but also play a role in teaching and education (14-16).

Participation in an EQA scheme improves between-laboratory performance and can act as a tool to identify problems experienced by the participating laboratory (17, 18). There are several well recognized international immune monitoring EQA schemes available for CD4 testing including UK NEQAS for Leukocyte Immunophenotyping (www.ukneqas.org.uk), The College of American Pathologists (CAP) (19) and HIV Immunology, Health Canada Quality Assessment Scheme International (QASI) (www.qasi-lymphosite.ca). In comparison with their international counterparts, relatively few laboratories in Africa (including Sub-Saharan Africa) participate in international EQA schemes.

In Africa in 2003, 51% of countries had a national quality assurance programme in place for CD4 testing, but participation was limited to the main reference laboratories. Further, the World Health Organisation (WHO), reported that EQA testing was conducted at least once a year in 53% of African countries in 2003, but only half of these laboratories actually submitted results (20). Problems associated with participation include, the high cost of EQA schemes, logistical problems with sample transport to testing sites and lack of infrastructure and computers to enter data onto websites for submission of results. In addition, international EQA schemes are frequently commercial investments and their focus may not necessarily be the development of quality laboratory capacity building in resource-poor settings. Promotion of locally or regionally supported EQA schemes would therefore have more impact in Africa if the scheme is run with an understanding of regional difficulties and circumstances, methods used and even local languages where applicable. AFREQAS (21) was introduced in 2002 in support of this need and initially

underwritten by WHO (22) and later supported through the South African National Health Laboratory Service (SA-NHLS) (23).

CD4 EQA schemes have used fresh whole blood and frozen cells, but these schemes are typically limited to countries like the USA and Europe, where transport infrastructure could support rapid transport of samples to testing sites under temperature controlled conditions. However, this situation is not ideal, as this material can generate wide coefficients of variation (%CV) and masked factors that affected a laboratory's performance (24). To overcome these problems, stabilized whole blood products were adapted by some (25, 26) for CD4 EQA schemes but not others (27). Use of stabilized whole blood enabled distribution of CD4 EQA material across countries and even continents. Further, inter-assay and inter-laboratory performance could be compared without being compromised by the instability of fresh samples during shipment and storage (25, 26). The disadvantage however, of stabilized blood is that it is not compatible with all types of haematology analyzers or CD4 methods and is therefore not recommended for dual platform CD4 analyses where an automated lymphocyte count is required for calculation of the absolute CD4 count (14, 28). The use with manual bead assays has also been difficult (29).

1.2 Background to the need to promote CD4 EQA for the Sub-Saharan Region of Africa

Sub-Saharan Africa remains the region most heavily affected by human immunodeficiency virus (HIV) (22 million), accounting for 67% of all people globally living with HIV and for 75% of HIV-associated deaths in 2007 (30). Estimates of the number of adults and children living with HIV/AIDS in South Africa were 5.35 million in 2008 (31). On average 1.9 million new infections are reported annually for this region of which most are in the age group 15-24 years. The global move to improved universal access to ARV therapy has resulted in major efforts toward making therapy cheaper and more readily available to Sub-Saharan Africa, with an estimated 2.3 million people on therapy in this region at the end of 2008 (26). Although financial aid to these regions has increased 6-fold since 2001, with \$10 billion US dollars spent on treatment and prevention of HIV in 2007, relatively little funding has been used to develop laboratory capacity. Although HIV

incidence seemed to have stabilized globally since 2000, the latest South African prevalence rate of HIV infection for the total adult population is 18%, (all age/sex groups) (31). Therefore, South Africa has urgently scaled up both HIV prevention and treatment programmes as well as providing infrastructure for ongoing monitoring of HIV patients on antiretroviral therapy (32-39).

1.3 The laboratory measurement of CD4

Although various laboratory tests are available for diagnosis and monitoring of HIV infection (rapid testing, ELISA testing and HIV-viral load by PCR) respectively (40), the absolute CD4 T-cell count has been established as the method of choice to stage and monitor the progression of HIV infection (41, 42). The normal reference interval for healthy adults in South Africa is an absolute CD4 count of 500-2010cells/ μ l and a CD4 percentage of lymphocytes of 27-58% (43). HIV infection causes a decline in the absolute and percentage CD4 positive cells as the disease progresses. Initiation of anti-retroviral treatment is recommended when the absolute CD4 count drops to below 350cells/ μ l, but in South Africa and other resource-poor setting, level of 200cells/ μ l have been advocated (44-46).

CD4 T-cell enumeration methods covers a wide spectrum of technology and skill requirements, ranging from simple manual assays (i.e. Dynabeads, TRAx CD4 Assay, Microvolume fluorimetry, Beckman Coulter Cytosphere system, Capcellia and dried blood spots) (40, 47-52) at the low end to automated flow cytometric testing at the high end of the spectrum (Beckman Coulter, Becton Dickinson, Partec) (53-58).

Table 1.1 outlines the different types of CD4 methodologies described for use in Sub-Saharan Africa. To date both flow cytometric and manual bead based, requiring a microscope, methods have been developed for CD4 enumeration. Typically, manual assays require minimal technical skills and flow cytometric systems require more technical skills. Flow cytometry is however considered the gold standard/reference method for the enumeration of CD4 counts due to its proven accuracy and precision (21, 58-69). Although previously complex, technically demanding, and

costly method, it has evolved in recent years driven by the HIV/AIDS pandemic (61) to affordable bench-top instruments that can be operated with minimal training and at low running costs (61, 70-78).

	Manual Assays		Automated Assays				
			Low-medium sample throughput			High sample throughput	
Assay and Manufacturer	Coulter® Manual CD4 Count Kit Beckman Coulter	Dynal® T4 Quant Kit Invitrogen	FACSCount Becton Dickinson	Partec CyFlow Partec	Pointcare Now Pointcare Technologies	Beckman Coulter XL (Flowcare, PLG/CD4 or Tetrachrome T-cells)	Becton Dickinson FACSCalibur (TruCOUNT MultiSET)
Instrumentation	Neubauer counting chamber and light microscope	Magnet Neubauer counting chamber. Light/fluorescence microscope	Dedicated CD4 Counters			Dedicated CD4 Counters with advanced applications available	
Assay Principle	Direct observation of Bead Rosetted Cells	Direct observation of Immuno – captured cells	Flow Cytometry			Flow Cytometry	
Detection system	Latex Beads conjugated to anti-CD4	Magnetic beads conjugated to anti-CD4 and CD8	Fluorochrome labelled anti-CD3, CD4 and CD8 (3-color) OR CD4, CD14, CD15 (CD4 test alone)	Fluorochrome labelled anti-CD4 and CD45	Fluorochrome labelled anti-CD4 with colloidal gold particles	PLG/CD4 (CD45 and CD4 fluorochrome labelled antibodies) T-cells (CD45, CD4, CD3 and CD8 fluorochrome labelled antibodies)	4-color (CD3/ CD8/ CD45/CD4 fluorochrome labelled antibodies) 3-color (CD3/CD4/ CD45 or CD3/8/45 or CD4/8/3 fluorochrome labelled antibodies)
Results	Absolute CD4 count	Absolute CD4 and CD8 counts	Absolute and % CD4 counts Absolute and % CD8 and CD3 counts			Absolute and %CD4 counts Absolute and % CD3 and CD8 counts	
Advantages	Simple and rapid	Simple and rapid	Simple with little operator intervention or skill Well quality controlled through both internal and external quality control schemes Some systems can operate on battery power			Cost efficient for high volume testing Instruments have additional applications Well quality controlled through both internal and external quality control schemes Reagents are generally less expensive Reagents can be used interchangeably between systems	
Disadvantages	Subjective Low throughput No external quality assessment programmes available because of the specific local methodology. These products have been reported as not compatible with stabilized blood products.		High cost of initial installation and maintenance Need good technical support Need trained/skilled laboratory staff Dedicated with limited applications Maximum 50-100 samples per instrument per day Reagents relatively expensive EQA material cannot be analyzed on all systems Have specific reagents and kits tailor made for the instrument			High cost of initial installation and maintenance Need good technical support Need trained/skilled laboratory staff Need good laboratory infrastructure (i.e. temperature control, dust free environment) Need uninterrupted power supply	

1.4 Implementing a CD4 EQA scheme for Sub-Saharan Africa (including South Africa)

The aim of this project was to review the first twenty trials of the regional CD4 AFREQAS scheme. This scheme was implemented to raise awareness of the need for quality laboratory testing and to continually improve and maintain a high standard of CD4 enumeration used in support of treatment programmes.

The aim of this study was sevenfold:

1. Establish the requirements of implementing a regional EQA scheme including all documentation, data collection platform and logistical aspects including transportation and methods of result submission and participant feedback.
2. Establish robustness of a commercial stabilised fixed blood product during the pilot initiation for use in subsequent trials.
3. Establish methods for data analysis of all submitted results and further establish a database for analysis of cumulative trial data.
4. Analyse the participation performance of all users, laboratories and subgroups defining all methods used within each trial.
5. Analyse the cumulative pooled SDI data across 20 trials to assess performance of subgroups of users using similar methods identifying deficiencies and outline systems best used by African users.
6. Review participant performance with respect to strengths and common errors, to ascertain areas for training initiatives.
7. Show by example which statistical analysis of comparative data, how intervention post-poor performance, can positively impact on an individual laboratory's ability to report reliable CD4 counts.

2.0 MATERIALS AND METHODS

2.1 Study design

This was a retrospective study where results were collected from laboratories in South Africa and Sub-Saharan Africa participating in the CD4 AFREQAS scheme. Results of the first 20 shipments (29 samples) were collected from July 2002 to September 2006. Absolute CD4 counts (abs counts) and CD4 percentage of lymphocytes (CD4%Ly) were statistically analysed for comparison of data with each trial and cumulatively across 20 trials.

2.2 Study Participation

National government CD4 laboratories, university laboratories and private pathology laboratories were included in the study. The CD4 AFREQAS scheme was initially implemented at local level, then put into operation at a national level, and finally extended to a regional African level.

The first CD4 AFREQAS trial was conducted in the Gauteng province, where thirteen laboratories from Johannesburg and Pretoria participated. These laboratories were chosen for the initial Trial 1 study as they performed high volumes of CD4 tests. Prior to participation, the CD4 AFREQAS scheme coordinator met with each participating laboratory manager to discuss the aim and benefits of the programme. The laboratory managers showed an interest in both internal quality control as well as external quality assessment and wanted to improve on their general laboratory performance. During the initial site visit, to these and subsequent participants, laboratories were made aware of the guidelines and standards available on Good Laboratory Practice (GLP) and Good Clinical Laboratory Practice (GCLP) to enable them to introduce GLP into their laboratories (1). Participants were further informed that results generated from their laboratories were in the interest of patient care, by generating reproducible results, to standardise techniques across centres and improve the CD4 laboratory diagnostic service.

The aim of CD4 AFREQAS was to provide an assessment scheme (advisory) and not an assurance scheme (policing). The CD4 AFREQAS scheme offered assistance to laboratories with regard to corrective actions but was not responsible for ensuring implementation of these advised corrective actions.

Following the Trial 1 pilot, Trial 2 was extended nationally and comprised laboratories from Bloemfontein, Durban and Cape Town, extending the scheme to a total of 25 participants. Trial 3 was extended regionally into Africa and laboratories from Namibia, Swaziland, Botswana, Mozambique, Angola, Zimbabwe, Senegal, Uganda, Ghana and Burundi participated (n= 44 participants). By September 2006 (Trial 20), Zambia, Kenya, Rwanda, Nigeria, Cote d'Ivoire, Lesotho, Germany and India had also joined the scheme.

The National Health Laboratory Service (NHLS) enrolled the national network of laboratories on the scheme from Trial 6 in 2004. In the first year of CD4 AFREQAS scheme, two trials were sent out followed by three in 2003 and four in 2004. From 2005, the frequency of trials sent out increased to six times a year, in the first week of every second month in line with the frequency of the UK NEQAS Immune Monitoring CD4 scheme (www.ukneqas.org.uk).

2.3 Documentation

Various documents were generated, both to ensure the success of the scheme and to obtain information and data required for this dissertation.

2.3.1 Registration form for participants

The registration form was the first form to be developed, as it was important to get clear information regarding each participant. The registration form was given to all new participants to supply details of a physical address for courier dispatch, a postal address for all correspondence, the name of the institution, an email address, phone number and fax number. All demographics on the returned registration form were collated into a Microsoft Excel software spreadsheet. As the study

progressed and more information became available, Microsoft Excel was not sufficient for data management and subsequently all information were transferred into Microsoft Access Software. Participation was deemed to be continuous with automatic annual renewal unless the participant advised the programme coordinator to the contrary in writing (Appendix B1).

To protect the confidentiality of each participant on enrolment, a unique participant number was assigned to each laboratory that was used across all trials (Appendix B2).

2.3.2 Certificate of participation

A certificate of participation on the CD4 AFREQAS scheme was given to participants annually, confirming registration and participation on the scheme for that year. Certification did not imply or ensure competence of participants as is the case with other international EQA programmes (18, 24). (Appendix B3).

2.3.3 Regional and International documentation

In addition to the registration form, the following documentation was required for participants outside South Africa.

2.3.4 Safety advice and instructions

A document was formulated for participants advising on the handling of the CD4 External Quality Assessment (EQA) material to be treated as potentially bio-hazardous. The WHO guidelines on HIV Safety precautions (WHO/EMC/97.3), as well as recommendations on safety practices in the laboratory (79), were distributed with each shipment (Appendix B4).

The instruction sheet gave general information on how to process the QC material, record results and a deadline was given for return of results (Appendix B5).

According to the manufacturer's instructions, the CD4 external quality assessment (EQA) material, which was fixed and stabilised, was to be treated as potential bio-hazardous material and instruction sheets carried an appropriate warning. The manufacturer states in the package insert that all blood used in the preparation of the EQA material was tested by the Food and Drug Administration (FDA) approved method for the presence of antibodies to Human Immunodeficiency Virus (HIV-1, HIV-

2), Hepatitis C Virus (HCV) and Hepatitis B surface antigen (HbsAg) and found to be negative (were not repeatedly reactive) (80). Because no test method could offer complete assurance that HIV, HCV, Hepatitis B Virus (HBV), or other infectious agents were absent, the EQA material was recommended to be handled at Bio-safety Level 2 as prescribed for any potentially infectious human serum or blood specimen.

2.3.5 Export and Import permits

The export permit had to comply with the Department of Health Regulation 2 and 3 of GN 2306 of 21 December 1920. In terms of this regulation, the Department of Health Regulations authorise the export of biological substance specimens for External Quality Assessment testing, from the Republic of South Africa. These specimens comprised fixed, stabilised (non-infectious) whole blood which was to be used in participating laboratories (Appendix B6).

Although the External Quality Assessment material had no commercial value, a description of the goods with a commercial invoice was required for customs purposes. Both the senders address (shipper), and recipients address (consignee) had to be stated. The commercial invoice had to be signed and dated, and the international courier waybill number entered on the invoice (Appendix B7).

In addition to the local material, stabilised whole blood supplied by Quality Assessment Scheme International (QASI), Ottawa, Ontario, Canada was donated for six trials namely; Trial 1, Trial 2, Trial 3, Trial 10, Trial 14 and Trial 16. An import permit was required from the South African Department of Health for the QASI material which had to comply with the Department of Health Regulation 2 and 3 of GN 2306 of 21 December 1920. In terms of this regulation, the Department of Health Regulations had authorised the import into South Africa of biological substance specimens for the CD4 External Quality Assessment programme. The material comprised fixed, stabilised (non-infectious) whole blood that could only be used in the External Quality Assessment programme (Appendix B8).

2.3.6 Data Report Form

Participants were given instructions on how to record their results as well as how to complete all the information required on the data form. This information was necessary to give a meaningful interpretation to the CD4 AFREQAS scheme.

2.3.6.1 Sample condition

Participants needed to comment on the sample condition, whether satisfactory or unsatisfactory. If the latter, they needed to give a reason (i.e. haemolysed, clotted, insufficient, etc)

2.3.6.2 Return Date

A date of submission of results was required to be entered by all participants.

2.3.6.3 Participant Name and Participant Code number

The participants were required to record their participant name and the participants' unique code number for identification.

2.3.6.4 Date of receipt and date of sample analysis

All participants were required to enter this information.

2.3.6.5 Antibody Panel Used

Participants needed to state the antibodies and fluorochrome combination used for CD4 testing as different antibodies and fluorochromes express varying levels of fluorescent intensity and are known to contribute to inter-laboratory variability (81, 82).

2.3.6.6 Flow cytometer used

Participants were given a selection of the instruments currently in use, i.e. BDS FACSScan, BDS FACSCalibur, BDS FACSCount, Beckman Coulter FC500, Beckman Coulter Elite, Beckman Coulter EPICS XL, Partec, Ortho Cytoron and Other (e.g. Guava). To assess the performance of groups of laboratories using the same methodology, it is important to determine which flow cytometer methodology is used for CD4 counting. Participants were also requested to give information on instrument maintenance and service frequency.

2.3.6.7 Flow cytometry platform used

Participants were required to state if the CD4 method used was dual platform or single platform.

2.3.6.8 Bead product

Participants were required to state the manufacturer of the bead product used (Flow Count™, Beckman Coulter or TruCOUNT™, Beckton Dickinson), if they reported single platform CD4 counts.

2.3.6.9 Lymphocyte Count Measurement

Participants using the dual platform method were asked to state the method used to obtain an absolute lymphocyte count.

2.3.6.10 Pipetting Method

Participants had to state if they used a manual or automated pipette and also if forward or reverse pipetting technique was used. They were asked to supply information on the calibration of pipettes.

2.3.6.11 Red-cell Lysis

Participants could choose between a no-wash no-lyse method (e.g. BDS FACSCount™) or a lyse-no wash method (e.g. ImmunoPrep or FACSLyse).

2.3.6.12 CD4 gating strategy

Participants were required to state the gating strategy used to identify CD4+ T-cells at the time of each shipment. The laboratory was required to choose the appropriate gating strategies, i.e. (i) Lymphocyte gating (FSC/SSC with CD3/4/8); (ii) CD45 bright lymphocytes (abbreviated version of CD3/4/8/45 guideline, 4 colour panel)(4); (iii) CD45 bright lymphocytes ((abbreviated version of CD3/4/8/45 guideline, 3 colour panel)(4); (iv) CD14/45 2 colour panel (6 tube, 2 colour, 1992 CDC guidelines) (46) or (V) PLG-CD4 total CD45 (83).

2.3.6.13 Workload

Participants were asked to state how many CD4 tests were processed per month in their laboratories.

2.3.6.14 Result Reporting

Participants were required to enter abs CD4 counts (CD8 and CD3 were an option but not reported in this study) results as cells/ μ l and CD4%Ly (CD8 and CD3 were an option but not reported in this study). NHLS laboratories were also required to submit their raw data for retrospective assessment of outlying performance, to identify sources of error and advise appropriate corrective action.

2.3.6.15 Comments or suggestions

Participants were given the option to enter comments and/or suggestions regarding the scheme as well as ask for advice on certain aspects of CD4 testing.

2.4 Preparation of EQA material for shipment

2.4.1 CD4 External Quality Assessment material (EQA) material

In addition to the Quality Assessment Scheme International, Health Canada (QASI) material, commercially available fixed, stabilised whole blood products that mimic laboratory whole blood CD4 enumeration procedures, were tested for suitability for distribution on the CD4 AFREQAS scheme. These products have known white blood cell concentrations and are supplied with reference ranges for both T-cell absolute counts (CD4, CD8 and CD3) and T-cell percentages (CD4, CD8 and CD3). The advantages of fixed blood preparations for CD4 EQA include wide compatibility with most flow cytometers, capacity to sustain cell integrity at 37°C (important for African distribution with no or little temperature control) and the ability to be used with various sample-processing procedures, such as lysing, fixing, washing, etc, (26). Initially, three commercial fixed, stabilised whole blood quality control products were assessed on Trial 1, namely, Cytocheck CD4 Low (Streck Laboratories, Inc), IMMUNO-TROL™ Normal (Beckman Coulter, Miami, FL) and IMMUNO-TROL™ Low (Beckman Coulter, Miami, FL). Upon analyses of the results from this trial, it was decided to use IMMUNO-TROL™ Normal and IMMUNO-TROL™ Low (Beckman Coulter, FL) for all subsequent trials. IMMUNO-TROL™ material was also supplied by QASI and

distributed in Trials 1, 4, 6, 10, 14, 17 and 18. On purchasing the CD4 EQA material the longest expiry date was requested to ensure the integrity of the samples for the dates of distribution.

2.4.2 Labelling of EQA material for distribution

EQA material was aliquoted into capped 2ml Sarstedt micro tubes (Biodex, Johannesburg). Labels were printed for each vial of quality control material distributed to participating laboratories, using the mini laser label (Avery Dennison Corporation, UK, Berkshire). Labels contained a sequential quality assessment number, for example, Trial 1 EQA 01.

2.4.3 Preparation of CD4 EQA material

The CD4 EQA material was stored at 2-8°C upon receipt from distributors. Care was taken to bring samples to room temperature (20-24°C) before aliquoting for distribution. On reaching room temperature samples were placed on a Stuart SSL4 mixer (Merck, Halfway House, Gauteng) for thorough, gently mixing to avoid no haemolysis. Once mixed, the material was pooled into a large sterile container and gently mixed again to ensure uniformity of aliquoted samples. The material was visually inspected for haemolysis by checking the plasma to see if it had a clear straw colour appearance as this would indicate no haemolysis, haemolysed red cells would give a red tinge to the plasma. A dark brown colour could be indicative of bacterial contamination. If material showed any discolouration, it was immediately discarded.

Strict safety procedures were in place for dispensing material in accordance with standard operating procedures of the flow cytometry laboratory. All work was carried out in a class II biohazard safety cabinet according to the manufacturer's instructions. A calibrated pipette was used to dispense 1ml of well-mixed CD4 EQA material into pre-labelled vials. After dispensing, the vials were capped and thoroughly checked for leakages before refrigerated until the dispatch date.

2.4.4 Validation of EQA material

Each batch of EQA material was validated using two CD4 testing methods by the CD4 AFREQAS coordinator after dispensing (before dispatch). Both single and dual platform PLG-CD4 enumeration was performed (79, 83, 84). These results were used to confirm the reference CD4 values given with the purchased quality assessment material (package insert). The same procedure was followed with the supplied QASI assessment material. These methods used a lyse, no-wash method (Beckman Coulter T-Q-Prep™ system) for sample preparation and the Epics®XL-MCL™ Beckman Coulter flow cytometer for analysis.

2.4.5 Dispatch of CD4 EQA material

For Trial 1, the CD4 EQA material was personally delivered by the CD4 AFREQAS coordinator (author of the dissertation) to each participant. Thereafter, the delivery of the CD4 EQA material was done through a courier service and the existing communication distribution network of the National Health Laboratory Service (NHLS). The CD4 EQA material that took longer than four days i.e. 4-6 days to be delivered, was sent in cooler boxes to limit temperature fluctuations. CD4 EQA material that was sent to Burundi, Senegal and Cote d'Ivoire took 4 days to reach their destinations. A delivery time of less than four days, that is 2-4 days, was sent at ambient temperature (18-22°) to, for example, Botswana, Swaziland, Namibia, Lesotho and Zambia. A good working relationship with the courier company was essential for the success of the programme, as the CD4 EQA material had to be delivered as quickly as possible to maintain the integrity of the material (85). The courier company supplied a daily update on time of delivery to each participating site and in this way; the transit time of each CD4 EQA sample was monitored. Table 2.1 gives a summary of the CD4 EQA material.

Table 2.1: Summary of CD4 EQA material dispatch

Trial number	Dates of sample dispatch	QASI	AFREQAS	Number of samples
Trial 1	2002 July	QASI		2
Trial 2	2002 October		AFREQAS	2
Trial 3	2003 July		AFREQAS	1
Trial 4	2003 September	QASI		1
Trial 5	2003 December		AFREQAS	1
Trial 6	2004 May	QASI		1
Trial 7	2004 July		AFREQAS	1
Trial 8	2004 September		AFREQAS	1
Trial 9	2004 November		AFREQAS	1
Trial 10	2005 February	QASI		2
Trial 11	2005 April		AFREQAS	2
Trial 12	2005 June		AFREQAS	1
Trial 13	2005 August		AFREQAS	1
Trial 14	2005 October	QASI		2
Trial 15	2005 November/December		AFREQAS	2
Trial 16	2006 January/February		AFREQAS	1
Trial 17	2006 March/April	QASI		2
Trial 18	2006 May		AFREQAS	2
Trial 19	2006 July		AFREQAS	1
Trial 20	2006 September	QASI		2

2.4.6 Courier collection

Once CD4 EQA samples were packed according to the above regulations, the courier company was informed and the consignments collected (86). A Monday was the preferred date for countries outside South Africa to ensure all participants received their CD4 EQA samples before the following weekend, avoiding delays of CD4 EQA samples at customs over the weekend as this would compromise the sample integrity. All African participants received their samples between two to four days after shipment. Tuesday was the preferred date for the South African distribution and the CD4 EQA samples typically reached the laboratories within twenty-four hours of dispatch.

2.4.7 Communication of dispatch

At the beginning of the year each participant was notified in writing of the dispatch dates. However, before a trial send-out, all participants were re-notified either by electronic mail (e-mail)/ fax/phone to expect sample delivery.

2.5 Compliance with international regulations for transportation and packaging of CD4 EQA samples

United Nations (UN) transport regulations were met when shipping CD4 EQA samples (87, 88). Biological substance specimens (CD4 EQA material) are assigned to Category B, or Risk Groups 2 and 3 and must comply with packaging instructions UN code 650 International Aviation Traffic Association (IATA safe transport of dangerous goods by air) (89) or Agreement of Dangerous goods by road and rail (ADR safe transport of dangerous goods by road and rail), assigned to UN code 3373 (90). The IATA air packaging instructions 650 had to be complied with for all air distributions in South Africa and Africa, whilst ADR road packaging instructions 3373 had to be complied with for all road distributions in South Africa only. Regional and International transportation required the relevant export, import permits and commercial pro forma invoices (87-89)

2.6 Pilot study to test sample stability

It was essential to establish the stability of the CD4 EQA material during transportation to the participating laboratories. The aim of the pilot trials was to assess the stability of the CD4 EQA material at ambient temperature (samples sent to Namibia and UK NEQAS laboratories in Sheffield, United Kingdom) and at 2-6°C (sample sent to Senegal).

In addition, further audits were performed with selected sites where each site received two sets of CD4 EQA material and were requested to return one set of material unopened to the NHLS CD4 reference laboratory. The returned CD4 EQA material was visually checked for haemolysis and

bacterial contamination. Samples were analysed by flow cytometry in the NHLS CD4 reference laboratory to ascertain that the T-cell subset enumeration was not adversely affected by sample transport.

2.7 Statistical analysis of CD4 AFREQAS results

Statistics were performed on two levels: (i) individual laboratory statistics and (ii) pooled statistics of all participants to assess the success of the EQA scheme. Both sets of statistics are reported to participating sites to allow bias estimation and comparison of individual performance against a pool of participants.

2.7.1 Software Packages used for data entry and analyses

Microsoft Word™, Microsoft Excel™, Analytical software STATISTIX 8, Graph Pad® Prism version 4, were used to generate statistics, graphs, documents, letters and feedback to all participating laboratories.

2.7.2 Statistical analyses of overall performance of all participants on the scheme

Each participant's data was analysed according to international practice (82, 91-93). On receipt of all participant data for each trial, CD4 abs counts and CD4%Ly values were entered into an Access database. The mean, standard deviation (SD) and coefficient of variation (%CV) was calculated automatically by the Access database program for the combined database of all results, and a $\pm 2SD$ limit determined for both CD4 parameters (untrimmed pooled data). Calculations were done in parallel on Excel spreadsheets for the first 10 trials. Results that fell outside the $\pm 2SD$ limit were identified and removed as outliers and pool data re-analysed to calculate a pooled trimmed mean, pooled trimmed SD and pooled trimmed %CV.

2.7.3 Statistical analyses of individual participant results for feedback reports

Each participant's residual value and standard deviation index (SDI) was calculated and compared to the pooled trimmed results (as described above) to indicate bias. Laboratories identified as outliers (outside of $\pm 2SD$ of the trimmed pool mean) were contacted to troubleshoot and perform corrective actions (94). These participants were not allowed to resubmit corrected results.

2.8 Return of data report form

Participants returned their results either by fax, NHLS internal postal service for NHLS participants or by e-mail. After statistical analysis and generation of individual laboratory reports, results were posted back to participants. However, this quickly proved inadequate, as the majority of the African participants either experienced delays in receiving their reports or did not receive their reports at all. This was resolved by sending the report of the previous trial with the subsequent CD4 EQA distribution. Participants were notified that a provisional report was available by fax or email if the participant needed the report before the next distribution. However, confidentiality was not guaranteed by this method of relaying results. Those participants whose results were outside the $\pm 2SD$ range or did not send in a return result were contacted immediately to effect corrective action timeously (Appendix B9).

2.8.1 Data capture and analyses of participant Data Report Forms

All relevant demographic and technical data was captured from the Data Report Forms onto ExcelTM spreadsheets at first and later onto a Microsoft AccessTM database. The participant information was used to assess factors that could impact results and result in bias or skewing of data due to the number of participants using a specific technique (i.e. SP versus DP) or methodology (i.e. BDS FACSCountTM versus PLG-CD4). The impact of each factor was calculated as the percentage of participant's versus the total group for each of the variables described under Materials and Methods 2.4.3.

2.8.2 Laboratory performance report

The performance report was the main interface with all the participants. For all trials the data was represented in tabular and graphic form. (Examples of these are given in Figures 2A and 2B). (Appendix B10).

2.8.2.1 Tables

As shown below (Figure 2.1A), the participant performance report included: the participants' laboratory name, the unique participant number, the region of the laboratory, the trial/survey number as well as the date of the trial, the lysing protocol, the instrument/flow cytometer and monoclonal antibody panel used.

The summary table of statistics was divided into two columns (Figure 2.1B). The first column reports the participant's results as abs counts and CD4%Ly. The 2nd column reports the results of all participants (global pool). The individual participants results reported for the individual participant included their reported results, the residual value (submitted result minus trimmed pooled mean result), and standard deviation index (SDI) (residual/SD of trimmed mean) with outliers flagged >2SD of the trimmed pool mean. The global pooled results reported the number of participants, the group trimmed mean value, standard deviation of the trimmed pool mean (SD) [$\sqrt{\sum(X_i - \bar{X})^2/(N - 1)}$] and percentage coefficient of variation (%CV) [SD/Mean x100].

A: An example of a laboratory's participation details



CD4+ Lymphocyte Proficiency Programme



Results of Survey number 4 of 2008 (QC026) Trial 31

Date of Report: 22 December 2008

Test Mean

LABORATORY: 022 - FLOW CYTOMETRY

REGION: CD4 REFERENCE LABORATORY

PROTOCOL: Lysed no Wash

INSTRUMENT: BCXL

MAb PANEL:

TUBE	Antibody 1	FL1	Antibody 2	FL2	Antibody 3	FL3	Antibody 4	FL4
1	CD45	FITC	CD4	PE				

B: An example of a participant's results

TEST	RESULTS	RESIDUAL	SDI	>+/-2,0	GLOBAL ALL TECHNOLOGIES				
					METHOD	No.	MEAN	SD	CV
1) CD4+ Lymphocyte Absolute count	164.00	-402.85	-7.41	*	ALL	247	566.855	54.377	9.59
4) CD4 % of Lymphocytes	26.80	-21.02	-7.22	*	ALL	191	47.822	2.912	6.09

Indicates an
outlying flagged
result
> ±2SDI

Participant's results

Pooled trimmed global results

Figure 2.1: Examples of tables used as part of the Laboratory Performance Report to indicate (A) participant details and (B) participant results.

CD4 methodologies were not separated and as such a participant's results were compared to the global pooled trimmed results for all methodologies on the scheme. Data was analysed and outliers identified and removed (trimmed data). The latter was analysed for mean, SD, %CV and SDI for each lymphocyte subset parameter reported. Individual participant results were compared directly to the trimmed SDI value, as recommended by international EQA programs (82, 91, 92).

2.8.2.2 Graphs

In addition to a tabled summary of participant's performance, results were also represented graphically (Figure 2.2). This graph represents a summary of SDI values for all participants, to help laboratories interpret their own results versus that of the pool. The limits (± 2 SDI) were highlighted to visualise outliers easily.

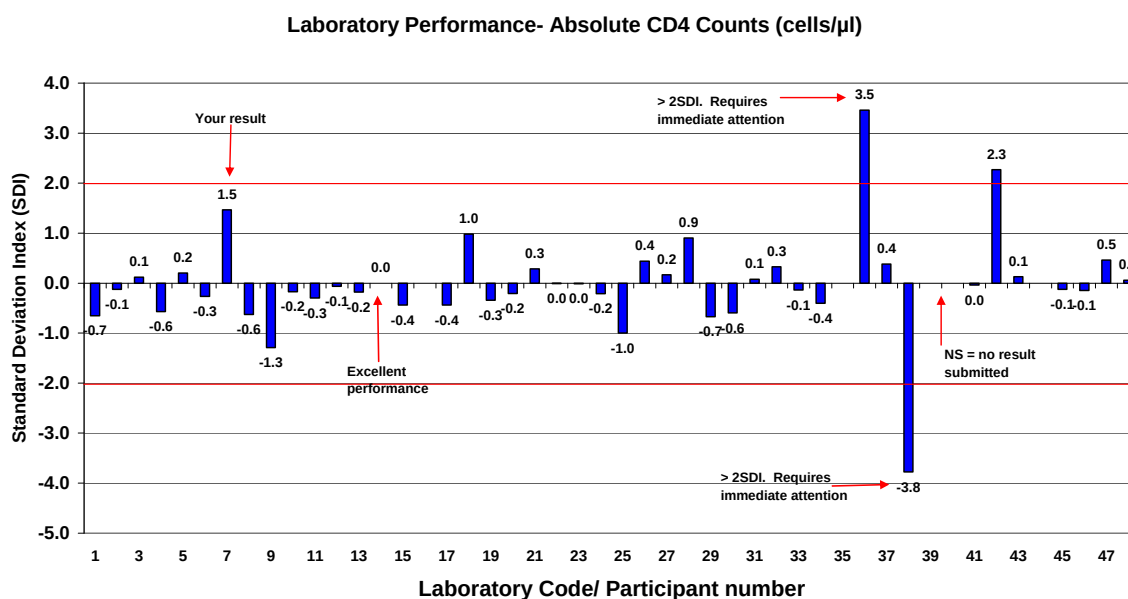


Figure 2.2 A bar chart depicting the participant laboratory number versus the calculated SDI value of individual participants. *Acceptable SDI limits indicated (red lines). Outliers are pointed out with red arrows. NS = no result submitted.*

Further, included in the result sheet was an explanation of how to interpret the graphs for laboratories that were not familiar with quality assurance procedures. In this context, as described by Lewis, a Standard Deviation Index (SDI) of less than 0.5 denotes excellent performance. A SDI between 0.5 and 1.0 is considered satisfactory. A SDI between 1.0 and 1.5 is considered acceptable, a SDI between 1.5 and 2.0 is considered borderline and lastly, a SDI of greater than 2.0 requires immediate attention and corrective action (85).

2.8.2.3 Monitoring own performance

Participant laboratories were provided with a blank Levey-Jennings wall chart to plot and monitor their performance over time (95). This was sent to the participants to use at their discretion for teaching and training purposes.

2.8.3 Feedback

On joining the AFREQAS programme, participants were made aware of the procedure of feedback, identifying problems and the infrastructure in place to assist laboratories with trouble shooting. These included training through one-on-one assistance (phone/fax/e-mail) and/or courses, help in identifying noted problems, validation of equipment, advice on different technologies and guidelines for equipment maintenance and regular service of instruments.

Participants who had problems with methods, instruments, trouble shooting poor results phoned, faxed or emailed the CD4 AFREQAS programme coordinator for assistance. Flow cytometry raw data was requested from sites to assist in troubleshooting.

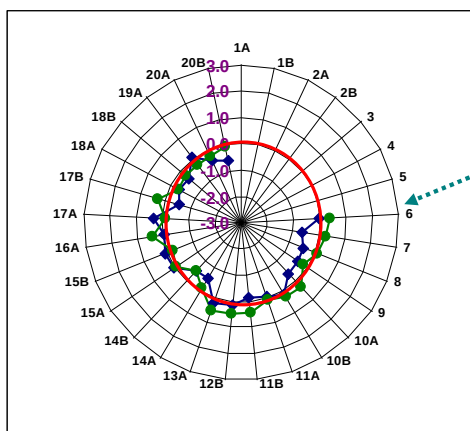
2.9. Retrospective assessment of serial longitudinal SDI follow-up performance for individual participants

For the purposes of this study, individual SDI performance of laboratories was monitored longitudinally by graphically representing the SDI values versus trial number on a radar graph (21). These radar plots enabled visual representation of the accuracy and precision of CD4 abs counts and CD4%Ly for individual participants.

The radar graphs depict the longitudinal follow-up of SDI values (Y-axis; range of -3 to +3) versus the trial number (X-axis; range of 1-20) (Figure 2.3). Radar graphs allowed visual representation of performance during consecutive trials in one snapshot view showing point of enrolment, non-submission of results or switched to alternative technologies. In addition this type of graph depicted improvement or problems in accuracy and precision over time by clearly indicating outliers.

The retrospective assessment of longitudinal SDI follow-up performance is also linked to demonstrating performance of laboratories with a network of users (e.g. Namibian Institute of Pathology national programme).

A: Laboratory with good performance over time



B: Laboratory with inconsistent performance over time

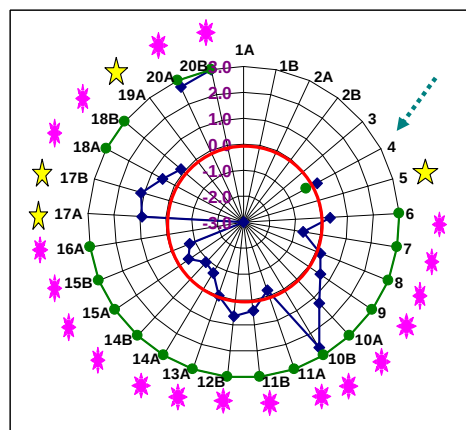


Figure 2.3: Examples of the use of radar graphs to evaluate participant performance over time (A and B). The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green ●) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. *Represents outliers, while ★ represents non-submission for a particular trial

2.9.1 Statistical analysis of overall precision of participant groups using the same methodology over 20 trials

After consultation with a statistician, within each of the twenty trials, separate sub-analyses were performed, to assess the between laboratory precision performance of laboratories using the same methodology. The most commonly used methodologies were assessed: BDS FACSCount™ (Becton-Dickinson, San Jose, California, USA), single platform Panleucogated CD4 (SP PLG-CD4) (Beckman-Coulter, Miami, Florida, USA), TruCOUNT™/MultiSET™ CD45 bright gated CD3/CD4/CD8 and Primary CD3 gated CD4/8 (Becton-Dickinson, San Jose, California, USA), and Partec® volumetric system (Partec®, Munster, Germany).

For each trial, submitted results for both abs counts and CD4%Ly for the above groups were pooled and a mean, SD and %CV calculated to assess accuracy and precision of each group. The

longitudinal precision of each methodology group used was analysed (%CV over the twenty trials) for both abs counts and CD4%Ly. Comparisons between methodology groups were done using 1 way ANOVA analysis. These results were plotted on scatter plots using GraphPad Software.

2.9.2 **Retrospective cumulative assessment of longitudinal precision of laboratories using the same CD4 methodology**

Outlier and non submission rates were calculated for each group of users and is shown in Table 2.2.

Table 2.2: Summary of outliers and non-submission, for the 4 major CD4 methods used by participants across the first 20 trials of the CD4 AFREQAS scheme.

Methodology	Absolute counts		CD4% Lymphocytes	
	% Outliers	% Non submissions	% Outliers	% Non submissions
BDS FACSCount™	2.8	9.9	NA	NA
BDS TruCOUNT™/MultiSET™	18.6	11.6	13.2	21.4
SP PLG CD4	6.4	5.5	3.4	4.1
Partec®	32.8	37.8	NA	NA

(NA = not applicable as these methods could not generate a %CD4 of lymphocytes at the time of the study).

To assess the longitudinal accuracy and precision of a group of laboratories using the same CD4 methodology (e.g. BDC FACSCount™), the standardised individual laboratory SDI values were pooled across the first 20 trials and grouped according to CD4 methodology used. Thus a mean (SDI) was calculated per user group per trial.

These mean (SDI) values were used in two ways.

(i) Radar plots show the mean of the pooled SDI values of a group of users, plotted trial by trial of a group of participants using the same methodology. (ii) The mean (SDI) values using STATISTIX 8 software were plotted using GraphPad Prism software in a Gaussian distribution plot. In this pooled SDI analysis grouped according to the same CD4 methodology, the calculated mean of the pooled SDI's, i.e., the mean (SDI) reflects longitudinal accuracy within a group of CD4 users. The SD (SDI) of the mean of the SDI namely, SD (Mean SDI), reflects longitudinal precision of a group of

CD4 users. In this analysis, ideal accuracy mean (SDI) values should be expected to be 0.0 with an expected range of -1 to +1. Ideal precision SD (Mean SDI) values should be expected to be 1.0 with an expected range of 0.0 to 1.0. Results were plotted as Gaussian distribution graphs for visual interpretation with acceptable ranges indicated and outliers identified as values outside these ranges.

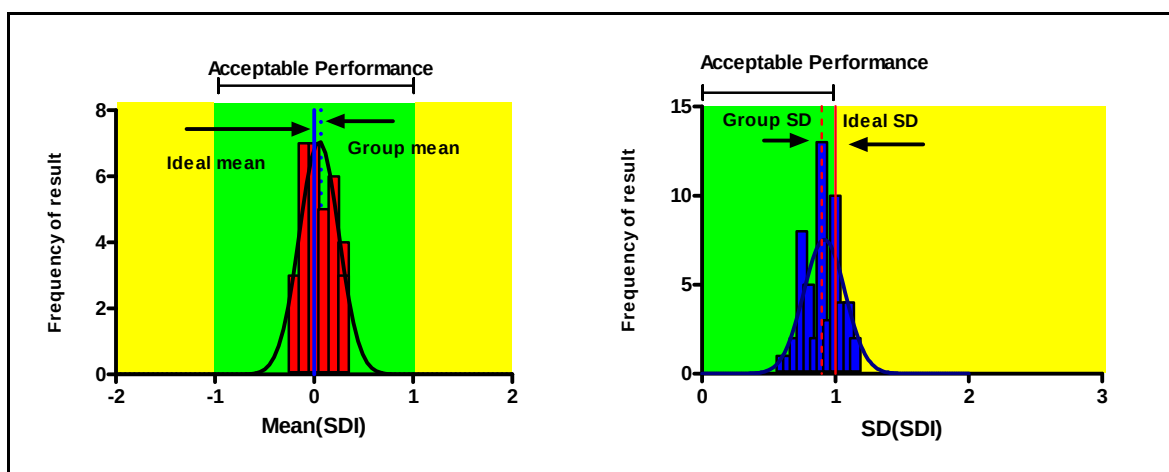


Figure 2.4: Examples of Gaussian distribution curves used to monitor performance of groups of technologies using the same CD4 method. Accuracy is measured as the mean of the SDI and precision as the SD of the SDI. *Acceptable performance is highlighted in green and poor performance in yellow.*

2.9.3 Within trial performance of five African laboratory networks and Grant supported networks over 20 trials

Five African countries that participated in the CD4 AFREQAS scheme had an established National Network of multiple laboratories for CD4 testing. These included The South African National Health Laboratory Service (NHLS), The Botswana National Government Laboratories, The Namibian National Institute of Pathology, The Lesotho National Government Laboratories and The Zimbabwean National Government Laboratories. The performance of laboratories participating within these national laboratory networks (South Africa, Botswana, Lesotho, Zimbabwe, Namibia) were analysed separately to assess accuracy and precision of national CD4 networks.

In addition to the five National programmes there were two networks with grant support. Significant training of personnel in laboratory management, laboratory methodology and principles

of good laboratory practice were conducted in these sites. Personnel were also given training in South Africa at the National Health Laboratory Service and Witwatersrand based Contract Laboratory Service as well through various commercial suppliers. Network A included laboratories in Kenya, Rwanda, Uganda and Zambia (9 sites), while network B included alternative laboratories from Kenya, Botswana, Uganda and Tanzania. The purpose of reviewing the performance of these groups was to ascertain impact of standardisation (or lack thereof), and further see if performance was improved or not, where training and resources were better (i.e. grant supported groups).

Pooled group mean, SD and %CV's were calculated and longitudinal performance measured by the mean %CV (precision) and mean SDI (accuracy) across 20 trials. Comparisons between groups were done using 1 way ANOVA analysis and results represented graphically as scatter plots of %CV results of 20 trials.

2.10 Investigation of laboratories with outlying results

This included error identification with appropriate feedback and corrective action investigation within a SA-NHLS laboratory and an investigation of an African laboratory with multiple outlying results.

2.10.1 Evidence of corrective action

This included error identification with appropriate feedback and corrective action investigation within a SA-NHLS laboratory.

First, an investigation to monitor the longitudinal maintenance of quality through participation on CD4 AFREQAS scheme was done. Secondly, an investigation was conducted into a laboratory within the NHLS, who encountered errors in the generation of CD4 absolute counts. This required the investigation of imprecision of pipetting through the evaluation of flow count rate monitoring

Validation of newly established NHLS CD4 laboratories comprised the following: Operator pipetting skill validation, instrument precision and accuracy validation and method validation. This validation is done by all new staff before moving onto the CD4 bench, to test their pipetting skills as well as the accuracy and precision of the pipettes used for the CD4 setup. It may also be used in the event of flow count rate discrepancies to eliminate faulty pipettes and/or operator skill as possible causes.

The use of Flow-Count™ (Beckman Coulter™) beads in the NHLS PLG-CD4 protocol is used to monitor the performance of the pipetting accuracy for each individual sample. This is a measure to ensure accurate quality reporting of the results. The absolute CD4 count is referenced to the beads (the calculation of the absolute CD4 count using beads is: $\text{CD4 events/bead events} \times \text{number of beads}/\mu\text{l} = \text{absolute CD4 count (cells/ } \mu\text{l})$). Any inaccurate added bead amount will impact on the accuracy of the single platform CD4 result. The NHLS use the flow count rate method for proactive quality control.

Monitoring the instrument performance and pipetting accuracy and precision over time as a quality control procedure is possible by plotting the flow count rate data. Monitoring of the flow count rate can be used to: monitor new technologist in training, assess the flow cytometer performance and assess pipette accuracy after pipette calibration (21, 96-99).

2.10.2 Investigation of a laboratory with multiple outlying results

To aid implementation of a more reliable technology in an African site with multiple outlying results participating on the CD4 AFREQAS scheme.

2.10.2.1 Assessment of bias of proposed new technology

A comparative study between their predicate method (BDS FACSCalibur MultiSET™) versus a proposed new technology (BDS FACSCount™) was performed. For this purpose, both local samples (n=43) and a panel of retrospective CD4 AFREQAS EQA samples (n=20) for assessment of precision on CD4 abs counts and CD4%Ly for accuracy assessment (May-June 2006). Fresh and CD4 AFREQAS samples were prepared and analysed at the site using both the reference instrument and the new instrument. The data was sent to the CD4 AFREQAS coordinator for statistical comparison. The following statistics were calculated for all parameters tested:

The minimum, maximum and median values (to indicate range of values included in the study), the standard deviation, and % similarity analyses $[\{a+b\}/2\}/a] \times 100$, where a represents the reference/gold standard methodology and b the test/new methodology) (100, 101) were also done. The co-efficient of variation was done on the CD4 AFREQAS samples as well. Results were plotted on scatter plots and Bland-Altman analyses done to detect bias (102).

3.0 RESULTS

3.1 Study Participation

The CD4 AFREQAS scheme expanded gradually over four year with three phases introduced. Phase one was at a local level of participation, phase 2 was at a national level and phase three at a regional African level of participation.

Since the inception of the CD4 AFREQAS in July 2002, the number of participants in the scheme had increased from 13 local South African sites to 195 sites (South African and rest of African laboratories) by Trial 20 in September 2006, representing an average annual growth rate of the scheme of 16.6% (Figure 3.1).

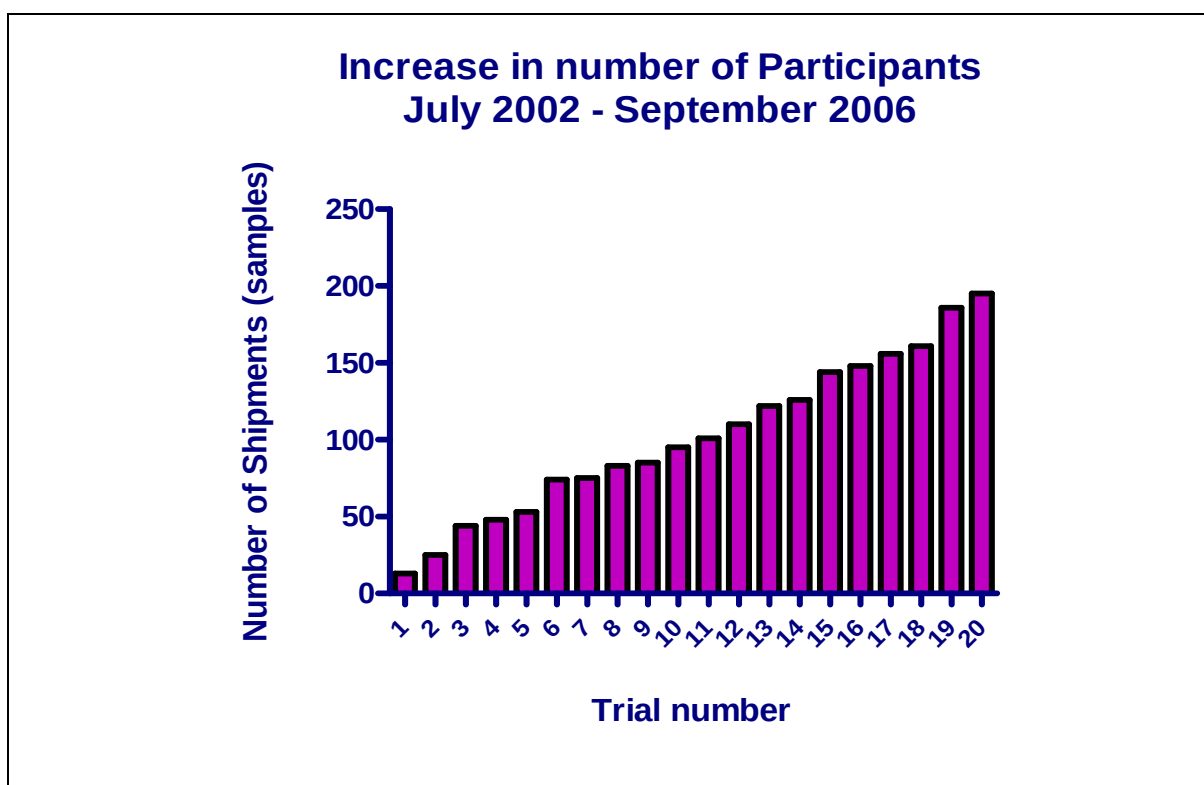


Figure 3.1: A graphical representation of the growth of the AFREQAS CD4 quality assessment scheme from July 2002 to September 2006 (Trial 1-20).

At the end of Trial 20 there were 195 participants from 20 countries, with full national CD4 programmes established in 5 countries including (i) South Africa (100 sites of which 40 were

NHLS laboratories, and the remainder consisting of academic/university laboratories, non-government laboratories and privately run laboratories); (ii) Namibia (6 laboratories, of which 5 are national government laboratories and 1 private laboratory); (iii) Lesotho (11 national laboratories); (iv) Botswana (15 laboratories of which 12 were national government laboratories and 3 private laboratories) and (v) Zimbabwe (19 national laboratories). There were an additional 4 countries which had a semi-developed network with more than 5 laboratories registered per country. These included Zambia, Kenya, Uganda and Malawi. All the remaining participating African laboratories comprised 1 to 4 sites/laboratories per country. A German and Indian laboratory joined the program due to their affiliation to associated African laboratories. A German mission group requested to join CD4 AFREQAS as an honorary participant to facilitate corrective feedback to their mission laboratories in Africa that used their Partec® instruments as well as to assess the use of the CD4 EQA material and the Partec® instruments. The two Indian laboratories joined CD4 AFREQAS due to their affiliation with a grant supported group based in South Africa. The United Kingdom National External Quality Assessment Scheme (UK NEQAS) joined CD4 AFREQAS as an independent individual participant.

3.1.1 Participant Response Rate

The CD4 AFREQAS scheme had issued 20 trials by the end of 2006, equating to 29 samples of which 20 were “normal” CD4 value material and 9 “low” CD4 value EQA materials. The response rate, defined as the number of returned results divided by the number of shipped samples multiplied by one hundred to obtain a percentage (range 86.0% to 100%), with an average response rate of 91.9% was noted (Figure 3.2). A summary of the user’s outlier and participation rates per trial is outlined in Table 3.1. (To view breakdown of non submissions and response in groups of laboratories using the same CD4 method see Table 3.2). Delivery by courier was highly reliable with no non-delivery of samples reported. Average sample non-return rate was 8.1%.

Table 3.1: Participant numbers, percentage increase, response rates and outlier rates over trials 1-20.

Phase	Level	Trial number	Shipped samples (n)	Percent increase since previous trial	Response rates (%)	Outlier rates (%)	
						CD4 abs Counts	CD4%Ly
One	Local	1A	13		92.9	7.7	0.0
		1B			92.9	0.0	7.7
Two	National	2A	25	78.6	100.0	4.0	12.0
		2B			100.0	4.0	12.0
Three	African	3A	44	76.0	100.0	4.2	8.3
		4A	48	9.1	89.6	7.0	5.4
		5A	53	10.4	92.5	4.1	2.3
		6A	74	39.6	100.0	4.1	4.7
		7A	75	1.3	88.0	3.0	5.5
		8A	83	10.7	91.6	3.9	6.3
		9A	85	2.4	89.4	5.3	4.8
		10A	95	11.8	89.5	3.5	9.0
		10B			89.5	3.5	4.5
		11A	101	5.9	89.1	4.4	7.6
		11B			89.1	2.3	6.3
		12B	110	8.9	90.9	3.0	6.5
		13A	122	10.9	86.1	3.8	4.9
		14A	126	3.3	92.9	6.0	6.6
		14B			92.9	4.3	7.7
		15A	144	14.2	89.6	6.2	6.2
		15B			89.6	2.3	3.1
		16A	148	2.7	92.6	3.6	4.8
		17A	156	5.4	93.6	4.8	1.8
		17B			93.6	4.8	2.7
		18A	161	3.2	91.3	2.0	4.3
		18B			91.3	3.4	3.5
		19A	186	15.5	86.6	3.7	4.3
		20A	195	4.8	92.8	5.5	5.5
		20B			92.8	5.0	3.1

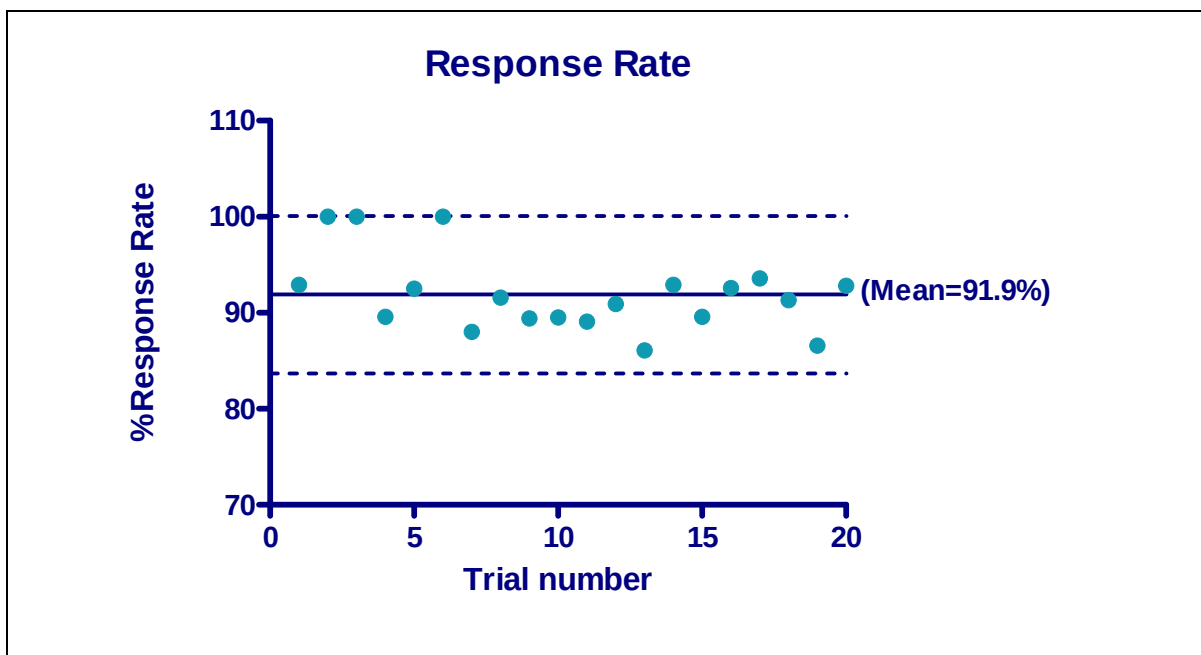


Figure 3.2: The percentage response rate of participants from Trial 1-20, showing a mean of 91.9%.

3.1.2 The percentage outlier rate

An outlier was defined as a submitted result falling outside of ± 2 standard deviation of the trimmed pool mean. The percentage outlier rate of absolute CD4 counts (Abs CD4) for all trials was 4.1% and CD4% of lymphocytes (CD4%Ly) for all trials was 5.6% (Table 3.1). The percentage outlier rate for normal CD4 EQA material was 4.5% for abs counts and 5.8% for CD4%Ly. The percentage outlier rate for low CD4 EQA material was 3.2% for abs counts and 5.0% for CD4%Ly.

The percentage outlier rate and non submission of the four most commonly used methodologies is summarized in Table 3.2 below.

Table 3.2: Summary of outliers and non-submission, for the 4 major CD4 methods used by participants, across the first 20 trials of the CD4 AFREQAS scheme.

Methodology	Absolute counts		CD4% Lymphocytes	
	% Outliers	% Non submissions	% Outliers	% Non submissions
BDS FACSCount™	2.8	9.9	NA	NA
BDS TruCOUNT™/MultiSET™	18.6	11.6	13.2	21.4
SP PLG CD4	6.4	5.5	3.4	4.1
Partec®	32.8	37.8	NA	NA

(NA = not applicable as these methods could not generate a %CD4 of lymphocytes at the time of the study).

Overall BDS FACSCount™ and SP PLG-CD4 users showed the least outliers and non submission of results across 20 trials with an average of <5% and <7% respectively. The BDS TruCOUNT™ MultiSET™ user group showed higher values across twenty trials with outliers and non submission of results with an average of <16% and <17% respectively. The Partec® user group had the highest outliers and non submission of results across 20 trials with an average of <33% and <38% respectively.

3.2 Summary of relevant information from participant Data Report Forms

3.2.1 Sample Condition

There was positive feedback on the sample condition. No samples were reported haemolysed, contaminated, clotted or insufficient across the 20 trials supporting the stability already described (26).

3.2.2 Submission of Results

All returned results were received by or before the due date. However, there were a few non submissions, mainly caused by technical problems encountered in individual laboratories.

3.2.3 Antibody Panel Used

Seventeen percent (17%) used 1 monoclonal antibody. This group included the volumetric Partec® based instrument and Guava® EasyCD4 users. Twenty seven percent participants used the PLG-

CD4 method with Beckman Coulter™ BCXL instruments, using 2 monoclonal antibodies. Eighteen percent used 3 monoclonal antibodies. This group comprised of BDS TC/MS Primary CD3 gating, BDS FACSCount™, FACScan™ and Beckman Coulter™ systems. Eighteen percent used 4 monoclonal antibodies. This group comprised of BDS TC/MS CD45 bright gating and Beckman Coulter™ Tetra one systems.

3.2.4 Flow Cytometer used

Forty six percent (46%) of participants used Beckton Dickinson of which 24% used FACSCalibur™ and 22% used FACSCount™ instruments. Forty one percent (41%) of participants used Beckman Coulter™ BCXL instruments. Ten percent of participants (10%) used the volumetric Partec® based instruments, while the remaining 3% of participants used FACScan™, BC FC-500 or Guava® EasyCD4.

3.2.5 Flow Cytometry Platform used

At Trial 20, ninety three percent (93%) of participants used single platform and 7 percent (7%) used dual platform methodology.

3.2.6 Bead Product Used

Fifty percent (50%) of participants, used Beckton Dickinson™ technology used FACSCalibur™ with TruCOUNT™ beads. Forty eight percent (BDS FACSCount™ users used bead product supplied in ready prepared kit). Ninety five percent (95%) of participants who used Beckman Coulter™ BCXL instruments used Flow Count™ beads. One percent Beckman Coulter™ FC-500 used Flow Count™ beads. These percentages were calculated from the information supplied by participants across 20 trials.

3.2.7 Pipetting method

All BDS FACSCount users™ used automated pipettes. Beckman Coulter™ BCXL, BDS FACSCalibur™, Partec®, FACScan™, BC FC-500 and Guava® EasyCD4 all used manual pipetting methods. Sixty percent (60%) of laboratories calibrated their pipettes either once or twice a year.

3.2.8 Red cell lysis method

From the information supplied by the participants, seventy seven (77%) percent of participants used lyse no wash methods; twenty three percent (23%) used a no wash no lyse method.

3.2.9 CD4 Gating Strategy

Gating strategies used by the participants are described in Table 3.3

Table 3.3 Summary of gating strategies used by participants

CD4 GATING STRATEGY	% Participants
Lymphocyte gating (FSC/SSC with CD3/4/8)	5
CD45 bright lymphocytes (abbreviated version of CD3/4/8/45 guideline)	10
CD45 bright lymphocytes (3- or 4 colour panel)	17
PLG/CD4 total CD45	37
BDS FACSCount	21
Partec volumetric	10

3.2.10 Workload of laboratory

Most participants did not state the number of CD4 samples tested per month. Feedback was not forthcoming from laboratories citing confidentiality constraints.

3.3 Assessment of CD4 EQA material

In Trial 1, two different commercial EQA materials were evaluated, by distribution to 13 participants on Trial 1 to ascertain which product was most suitable for future trials. The products assessed were IMMUNO-TROL™ Normal and Low (Beckman Coulter, Miami, FL) and Cytocheck CD4 Low (Streck Laboratories, Inc). These results of this analysis are shown in Table 3.4.

Table 3.4: Commercial EQA material

		ABSOLUTE CD4 COUNTS			CD4 % OF LYMPHOCYTES			
Immuno-Trol™ Beckman Coulter, Miami, FL								
Trial number	Pool mean	SD	%CV	N(labs)	Pool mean	SD	%CV	N(labs)
1A	604.9	60.8	10.1*	13	48.3	11.5	23.9	13
1B	146.8	28.4	19.3	13	16.0	8.3	51.9*	13
	Mean %CV		14.7		Mean %CV		37.9	
Precision performance using same material								
Cytocheck CD4 Low Streck Laboratories, Inc								
QC A	223.6	68.1	30.5*	13	16.1	7.8	48.3*	13
QC B	231.2	76.5	33.1*	13	16.2	8.5	52.6*	13
	Mean %CV		31.8		Mean %CV		50.5	

(* Outlier $\geq \pm 2SD$ identified, but not removed for statistical analysis) due to the low number of participants. This outlier identified came from a laboratory that incorrectly reported CD4%Ly of T cells instead of the required CD4% of lymphocytes.

Outliers were identified, but not removed from this pilot study according to the Dixon “Q”- test for removal of outliers on small sample number (103). Tighter between-laboratory precision was demonstrated with Immuno-Trol™ Normal and Low. A mean %CV of 10.1 for IMMUNO-TROL Normal and 19.3% for IMMUNO-TROL Low absolute CD4 counts were found vs. a mean %CV of 31.8% for Cytocheck CD4 Low control. Similar results were obtained for the CD4% of lymphocytes, with a mean %CV of 23.0% for IMMUNO-TROL Normal and 51.9% for IMMUNO-TROL Low vs. 48.3% and 52.6% for CytoChecks CD4 controls (Table 3.4).

The Cytochecks CD4 controls were not used after this initial pilot study as Immuno-Trol controls showed better overall precision between laboratories and were deemed to be the better product for distribution, in line with previously published works (26).

3.4 Pilot study to test sample stability

Table 3.5: A summary of trimmed SDI values for CD4 abs counts and CD4%Ly stability of EQA material at ambient temperature and 2-8°C

CD4 AFREQAS Pooled Trimmed SDI Results					
		Abs counts		CD4%Ly	
Site	Method	Pre audit	Audit	Pre audit	Audit
Senegal (2-8°C)	BDS FACSCCount	0.2	0.2		
UK NEQAS (ambient temp)	SP PLG/CD4	0.9	1.1	0.5	0.6
Namibia (ambient temp)	SP PLG/CD4	0.3	0.4	0.4	0.8

As shown above, transport and temperature did not affect EQA material stability in this audit. Samples tested at ambient temperature were in transit for 7 days (South Africa to the UK NEQAS laboratories in Sheffield, United Kingdom and back to South Africa NHLS CD4 reference laboratory), while samples shipped to Namibian laboratories and back to South Africa NHLS CD4 reference laboratory, took 5 days at ambient temperature. Samples to Senegal at 2-8°C took 14 days.

Additional audits were performed with selected sites where each site received two sets of CD4 EQA material and were requested to return one set of material unopened to the NHLS CD4 reference laboratory (n=46 samples tested).

Good accuracy and precision for abs counts and CD4%Ly was reported in keeping with the first audit. The mean abs counts noted to be 642 pre-shipment versus 643 post shipment, with a mean CD4%Ly 45.3 values noted both pre and post shipment (Figure 3.3).

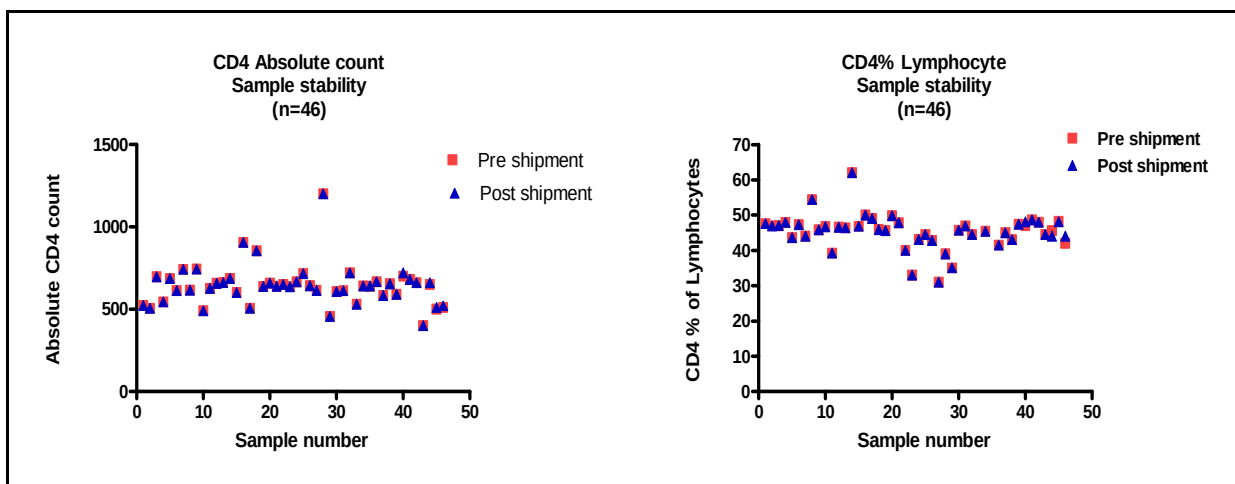


Figure 3.3: Comparison of CD4 abs counts and CD4%Ly used for sample stability audit study at ambient temperature.

3.5 Assessment of overall performance of all participants on the AFREQAS scheme

A summary of the statistical analyses of trimmed pooled data for all participants (irrespective of methodology used) is shown in Table 3.6.

Over the twenty trials, the trimmed mean %CV (between laboratory precision) for CD4 abs counts was 11.9%, ranging between 7.4% (Trial 13A) to 22.7% (Trial 4). The trimmed mean %CV (between laboratory precision) for CD4%Ly was 10.8% ranging between 4.4% (Trial 20A) to 51.9% (Trial 1B). For the normal level CD4 EQA material, the trimmed mean %CV (between-laboratory precision) for CD4 abs counts was 10.9% and 8.1% for CD4%Ly. For the low level CD4 EQA material analysis, the trimmed mean %CV (between-laboratory precision) for CD4 abs counts was 14.2% and 17.0% for CD4%Ly. Overall the participating laboratories showed poorer precision (%CV) with “low” material than with “normal” material for both CD4 abs counts and CD4%Ly. Accuracy was however relatively consistent between normal and low EQA material. Some trials (1A, 1B, 4, 5, and 15B) showed poorer accuracy and precision but were not removed from the final analysis. Specifically, the results for Trial 1 were included despite small participant numbers (and that data was not trimmed). Removing these trials from analyses, the %CV for the normal material absolute CD4 changed to 9.9% and normal CD4%Ly to a CV of 7.2%. For the low EQA material, the %CV for CD4 abs counts stayed at 14.2% while the CD4%Ly CV changed from 17 to 12.6%.

Table 3.6: Within trial performance of CD4 AFREQAS participants trimmed results of all methodologies, showing precision between participants.

Trial number	ABSOLUTE COUNT				CD4% OF LYMPHOCYTES			
	Mean	SD	CV	Participant (n)	Mean	SD	CV	Participant (n)
1A	604.9	60.8	10.1	13	48.3	11.5	23.9	13
1B	146.8	28.4	19.3	13	16.0	8.3	51.9	13
2A	609.3	66.2	10.9	25	41.7	2.2	5.3	25
2B	527.9	55.0	10.4	25	43.2	2.7	6.2	25
3	624.5	84.1	13.5	48	45.8	3.0	6.6	46
4	662.0	150.4	22.7	43	45.0	5.9	13.1	37
5	657.9	111.5	16.9	49	44.1	3.8	8.6	43
6	568.4	54.9	9.7	79	45.0	2.2	4.9	64
7	588.0	49.1	8.4	66	45.2	2.8	6.2	55
8	620.1	68.3	11.0	76	48.2	3.7	7.7	64
9	631.8	55.4	8.8	76	49.0	6.2	12.7	62
10A	626.0	60.1	9.6	85	43.8	2.2	5.0	67
10B	160.3	15.4	9.6	85	35.3	4.7	13.3	67
11A	637.2	52.8	8.3	90	44.9	2.5	5.6	66
11B	137.2	19.6	14.3	88	19.7	2.2	11.2	64
12B	163.8	20.0	12.2	100	34.8	3.3	9.5	77
13A	590.4	43.6	7.4	105	43.0	3.7	8.6	81
14A	496.9	52.9	10.6	117	42.2	4.5	10.7	91
14B	146.2	20.9	14.3	117	18.5	2.1	11.4	90
15A	481.7	49.4	10.3	129	42.2	4.2	10.0	97
15B	144.0	24.4	16.9	128	19.1	4.3	22.5	96
16A	536.7	48.4	9.0	137	43.9	2.8	6.4	104
17A	537.0	44.4	8.3	146	40.1	1.8	4.6	111
17B	146.5	11.7	8.0	145	21.3	2.1	9.7	110
18A	515.8	59.8	11.6	147	40.3	2.2	5.5	115
18B	141.7	19.1	13.5	146	21.0	2.2	10.5	114
19A	529.7	55.2	10.4	161	40.3	2.2	5.5	115
20A	685.4	67.7	9.9	181	45.0	2.0	4.4	127
20B	128.5	25.8	20.0	179	18.9	2.4	12.8	127
Mean	449.9	50.9	11.9		37.4	3.6	10.8	

(*Shaded cells indicate trials with higher trimmed %CV)

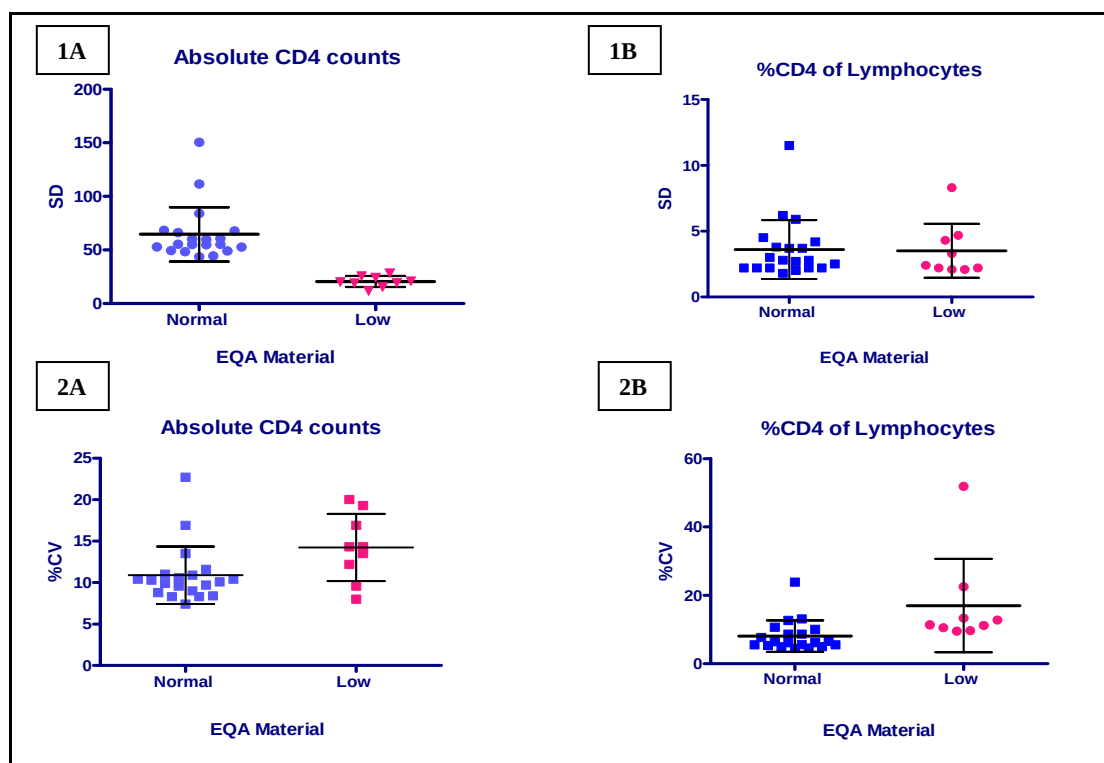


Figure 3.4: Comparison of SD (1A and 1B) and %CV (2A and 2B) values of the first 20 CD4 AFREQAS trials for both normal and low material, with no outliers removed. Each dot represents either trimmed SD or %CV from a single trial.

3.6: Summary of overall performance of all participants

Participant performance was characterised as excellent, good, and intermediate or poor based on the number of outliers and non-submissions. Excellent performers had neither outliers nor non-submissions during their time of participation. Good performers had 2 or less outliers/non-submissions, intermediate performers had 3-5 outliers/non-submissions and poor performers more than 6 outliers/non-submissions for the duration of their participation on the CD4 AFEQAS scheme. For intermediate and poor performing laboratories, a percentage improvement over time was calculated to assess if CD4 AFREQAS intervention had a positive effect on EQA results.

Table 3.7: Summary of the overall performance of individual participants and percentage improvement noted post input from CD4 AFREQAS scheme

Performance of total participants across twenty trials			
	Outliers and non submissions	Absolute counts	CD4 % Lymphocytes
Excellent	None	35.8%	40.6%
Good	< 2	30.8%	32.4%
Intermediate	3-5	21.4%	13.0%
Poor	≥ 6	11.9%	14.0%

3.6.1 Performance of laboratories reporting CD4 absolute counts

Overall, 35.8% of participants show excellent serial continued performance across 20 trials. Here the methodologies used comprised mostly SP PLG-CD4 and BDS FACSCount™ users. A single laboratory using the BC Tetrachrome™ four colour test was also included in this group.

A second group of users defined as good performers comprised a further 30.8%. Here, methodologies used including BCI Tritest™ primary CD3 gated, BDS FACSCount™ and SP PLG-CD4 users.

The third group of users, with 3 to 5 either outlying or non submitted results, comprising 21.4%. Users of BDS TC/MS and Partec® dominated this group.

Also the fourth and final of poor performers comprising 11.95% consisted of laboratories using mainly BDS TC/MS and Partec® systems. A single laboratory using DP Primary CD3 gating also performed very poorly and was included in this group. Of note, was the performance of the Zimbabwean group (see section on performance of National programmes), where limited reagents, maintenance and in some instances local politics, severely hampered adequate participation on performance.

3.6.2 Performance of laboratories reporting CD4%Ly

Overall, 40.6% of participants show excellent serial continued performance across 20 trials. Here the methodologies used comprised mostly SP PLG-CD4 users. A single laboratory using the BC Tetrachrome™ four colour test was also included in this group.

A second group of users defined as good performers comprised a further 32.4%. Here, methodologies used including BCI Tritest™ primary CD3 gated and SP PLG-CD4 users.

The third group of users, with 3 to 5 either outlying or non submitted results, comprised 13.0%. Users of BDS TC/MS dominated this group.

Also the fourth and final of poor performers comprising 14.0% consisted of laboratories using mainly BDS TC/MS systems. A single laboratory using DP Primary CD3 gating also performed very poorly and was included in this group. Of note, was the performance of the Zimbabwean group (see section on performance of National programmes), where limited reagents, maintenance and in some instances local politics, severely hampered adequate participation on performance.

To assess whether after in the groups defined as intermediate and poor performers, a further analysis of the group was undertaken.

In the group with intermediate performance, majority showed random as opposed to consecutive outliers, 65% participants, after serial outliers or non submitted results, showed improvement with time in this group.

In the group defined as poor performers, once again, the majority also showed random as opposed to consecutive outliers. Here only 25% of participating laboratories showed improved performance during Trials 17 to 20.

3.7 Assessment of individual participant results

3.7.1 Assessment of feedback reports

TE ST	RESULTS	RESIDUAL	SDI	>+/- 2,0	GLOBAL ALL TECHNOLOGIES				
					METHOD	No.	MEAN	SD	CV
1) CD4+ Lymphocyte Absolute count	164.00	-402.85	-7.41	*	ALL	247	566.855	54.377	9.59
4) CD4 % of Lymphocytes	26.80	-21.02	-7.22	*	ALL	191	47.822	2.912	6.09

Figure 3.5 Feedback report

For each participant the residual value (difference between participant result and pooled trimmed mean) was calculated. Here a negative value implies that the participant was reading lower than the pool mean, while a positive residual value indicated that the participant read higher than the pooled mean. In Figure 3.5 above, the participant showed a negative residual for both CD4 abs count and CD4%Ly.

The standard deviation index (residual/SD of trimmed mean expressed as a ratio) in the example above were outside the acceptable ± 2 SDI limit (as indicated by the asterix). A flagged result automatically raised a need for a corrective action for the laboratory concerned.

3.7.2 Retrospective assessment of serial longitudinal SDI follow-up performance of individual participants

The results below are examples of good versus a poor performing laboratory (irrespective of methodologies used) for the first 20 CD4 AFREQAS trials. Radar graphs (e.g. Figure 2.3 materials and methods) were generated to view serial longitudinal performance for each participating laboratory to identify outliers ($>\pm 2$ SDI), non-submissions of results where technology was switched. These graphs enabled graphic representation of laboratories showing good consistency and accuracy (Figure 2.3A) of CD4 abs count and CD4%Ly reporting. These radar graphs also showed poor accuracy and precision (Figure 2.3B). Due to confidentiality and objectivity and to ensure that all technologies were reflected fairly, no pre-selection of individual participant graphs are represented in this study. However, selected examples were used to illustrate problems (refer to section Appendix D, Supplementary data on troubleshooting and performance of individual laboratories).

3.8 Assessment of precision of participant groups using the same single platform methodologies within individual trials

For each trial, the %CV was plotted (Y-axis) against the trial number (X-axis) per methodology used. For trial 1 and 2, only BDS TruCOUNT™ MultiSET™ was performed as a single platform test. Dual platform methodologies (7% of total participants) were insufficient and excluded from this comparison. Single platform PLG-CD4 and BDS FACSCCount™ users started to participate from trial 3 onward, while Partec® joined the scheme from trial 8.

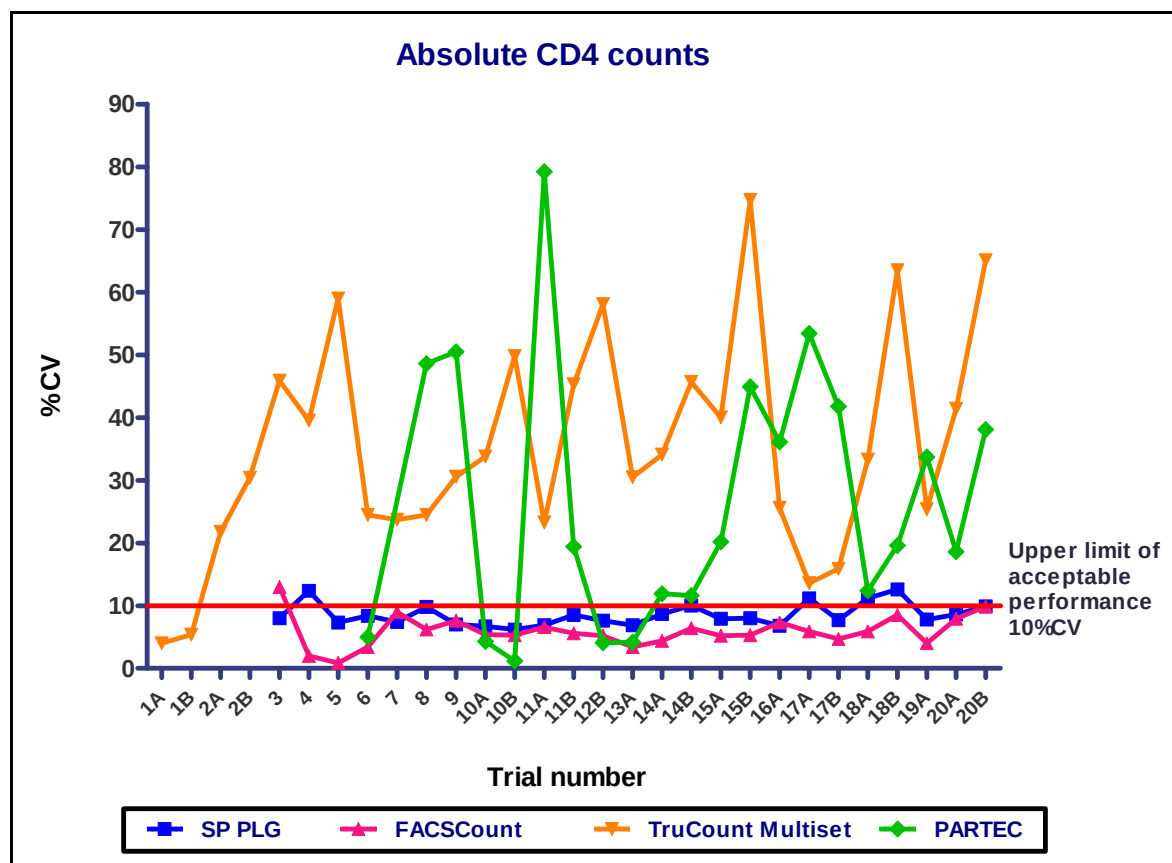


Figure 3.6: Between laboratory precision of laboratories using the same CD4 methodology for CD4 absolute counts over 20 trials.

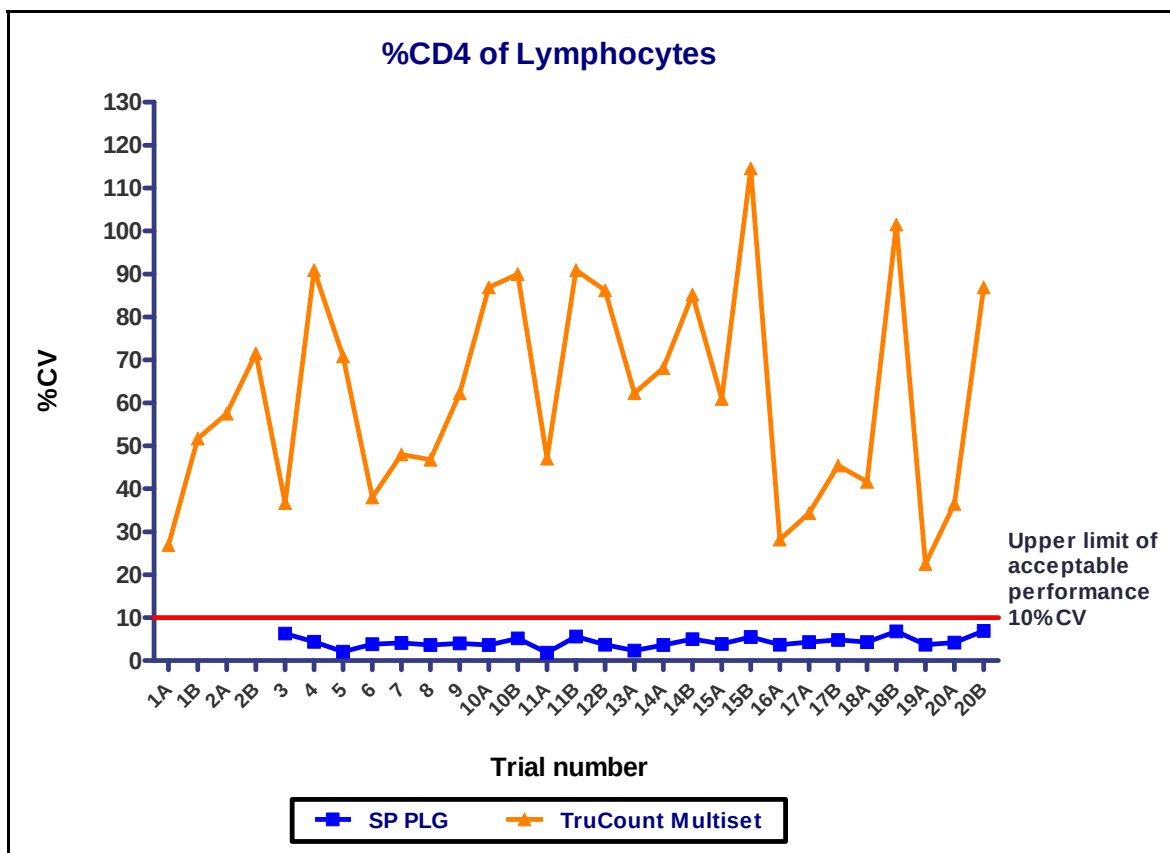


Figure 3.7: Between laboratory precision of laboratories using the same CD4 methodology for CD4%Ly over 20 trials. *BDS FACSCount™ and Partec® methodology cannot report CD4%Ly.*

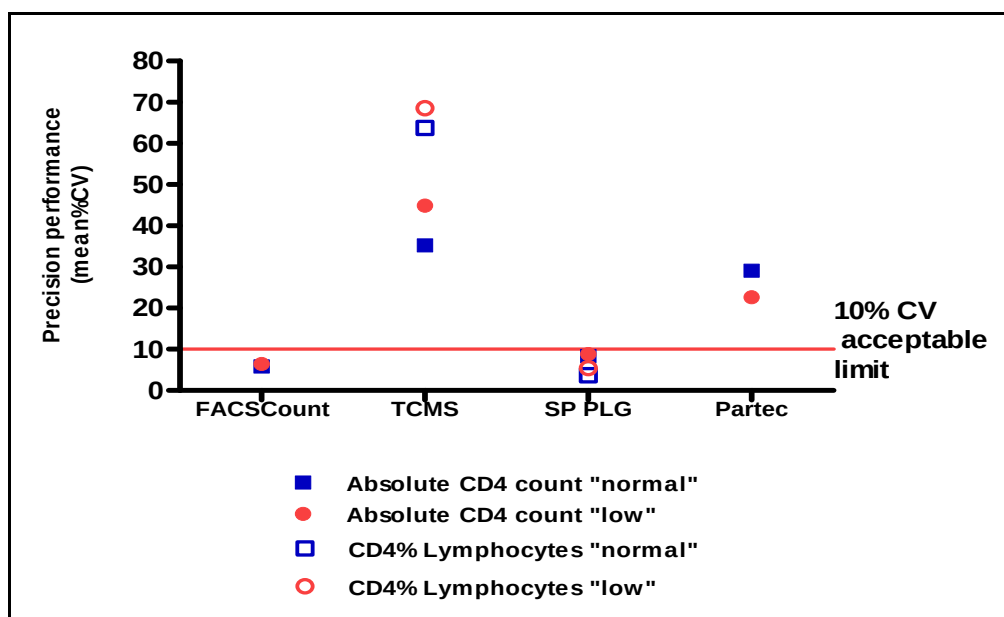


Figure 3.8: The average precision (mean %CV of 20 trials) of laboratories using the same methodology, split into normal and low levels of EQA material. *BDS FACSCCount™* and *Partec®* methodology cannot report CD4%Ly.

3.8.1 BDS FACSCCount™ methodology

Across the twenty trials, the performance of the BDS FACSCCount™ participants (comprising 22% of participants) for CD4 abs counts showed consistently good performance (BDS FACSCCount™ users could not report a %CD4 of lymphocytes) (Figure 3.6 and 3.8). The overall mean precision (%CV) was 6.0%. In trials where normal CD4 value material was shipped a trimmed mean %CV of 5.8% was recorded and trials where low CD4 value material was shipped, a trimmed mean %CV of 6.4% were noted. Poor precision was only noted in Trial 5 where a %CV of 61.8% was recorded for abs counts. This was due to a single outlier where one participant reported a CD4 abs count of 908 cells/μl (pool mean 657.8 cells/μl). On removing this outlying result (DIXON Q-test of outliers applied), within this group the trimmed mean for all BDS FACSCCount™ users was 657.8 with a %CV reduced to 0.9%CV.

Although BDS FACSCCount™ users could not report a CD4%Ly, 7.2% of BDS FACSCCount™ users incorrectly reported a CD4% of T-cells in place of the CD4% of lymphocytes. Such incorrect values were submitted for trials 2, 3, 5, 10-18 but in most instances, the participating BDS

FACSCount™ users understood the difference post intervention and did not repeat the error. However, these errors did not significantly impact the overall performance of CD4% Ly.

3.8.2 BDS TruCOUNT™/MultiSET™ methodology

Across the twenty trials the performance of participants that used the algorithm based BDS MultiSET™ system did not perform as well as the counterparts BDS FACSCount™ participants (comprising 24% of participants) (Figure 3.6 to Figure 3.8).

The overall mean between-laboratory precision (%CV) across twenty trials for both normal and low EQA material tested in laboratories using TruCOUNT™ MultiSET™ CD45 bright gated CD3/CD4/CD8 (TC/MS) showed a %CV of 22.4% and 33.3% for CD4 abs counts and CD4%Ly values respectively. In trials where normal CD4 value material was shipped a trimmed mean %CV of 18.7% and 27.3% for CD4 abs counts and CD4%Ly values respectively. In trials where low CD4 value material was shipped, a trimmed mean %CV of 30.7% and 46.6% for CD4 abs counts and CD4%Ly values respectively were noted.

Becton Dickinson manufactures two TruCOUNT™ Multiset™ CD4 enumeration kits; CD4/8/3 and CD3/8/45/4. Participants that used the latter, with CD45 bright gating, performed better (%CV of 14%) than participants that used a primary CD3 (CD3/CD4/CD8) gating protocol (21.4%). Laboratories using TC/MS and Primary CD3 gating showed an overall (all EQA material tested) %CV of 21.4% and 41.7% for abs counts and CD4%Ly respectively. In trials where normal CD4 value material were shipped a trimmed mean %CV for absolute CD4 counts and CD4 % of lymphocytes was 18.3% and 34.3% respectively. Low CD4 value material showed a %CV for absolute counts and CD4 % of lymphocytes was 28.3% and 58.0% respectively. In this group many participants incorrectly reported the CD4% of T-cells (CD3) instead of the CD4% of lymphocyte values. These transcription errors outliers impacted negatively on the overall precision of the MultiSET™ users group with respect to their CD4%Ly reporting. Participants using TruCOUNT™

MultiSET™ generally showed worse performance with low level material regardless of gating strategy used.

3.8.3 Single platform Panleucogated CD4 (SP PLG-CD4) methodology

Across 17 (Trial 3-20) trials the performance of laboratories using the SP PLG/CD4 participants showed consistently good performance (comprising 37.9% of participants) (Figure 3.6 to Figure 3.8).. Single platform PLG-CD4 was introduced into the NHLS from 2004 (Trial 3). Prior to this, PLG-CD4 was done as a dual platform method across three sites. Analysing the performance of laboratories using SP PLG-CD4, the overall mean %CV (precision) between laboratories was 8.5% and 4.3% for CD4 abs counts and CD4%Ly values respectively. In trials where normal CD4 value material was shipped a mean %CV was 8.2% and 4.2% CD4 abs counts and CD4%Ly values respectively. Where low CD4 EQA material was used a mean %CV was 8.8% and 5.4% CD4 for abs counts and CD4%Ly values respectively. Some participants (1.7%) incorrectly reported the %CD4 of the white cell count (%CD4 of PLG) instead of the required %CD4 of lymphocytes. However, these errors did not significantly impact the overall performance of CD4% of lymphocytes for this group.

3.8.4 Partec® methodology

Across 12 trials (Trial 8-20) the performance of laboratories using the Partec® methodology showed consistently poor performance (comprising 10% of participants) (Figure 3.6 and 3.8). Over the 12 trials of participation, a mean %CV (precision between laboratories) of CD4 for abs counts was 26.6%. Laboratories using the Partec® methodology are unable to generate CD4% of lymphocytes, nevertheless it generates a %CD4 of T-cells (CD3), similar to BDS FACSCount™. This value is however not the relevant clinical value typically required. Some Partec® users incorrectly reported CD4% of T-cells. However, on Trial 18, with corrective action feedback, this error was not repeated on subsequent trials. In trials where normal CD4 EQA material was shipped,

mean %CV (precision between laboratories) of 29.1% was recorded. Likewise, where low CD4 value material was shipped a mean %CV (precision between laboratories) of 22.6% was noted.

3.9 Retrospective cumulative assessment of longitudinal precision of laboratories using the same CD4 methodology

To compare performance between groups using the same CD4 methodology, individual laboratory SDI values were pooled across 20 trials and grouped according to methodology used. Separate analysis were performed overall and for normal and low EQA material data. All submitted results (including outliers) were used.

Within each group of CD4 users the first analysis plots the Mean SDI, trial by trial, in a radar plot distribution (Figure 3.9) The four main methodologies (BDS FACSCount™, BDS TruCOUNT™ MultiSET™ and SP PLG-CD4 and Partec®) are used in this analysis.

The second analysis plots these same Mean SDI values but instead in Gaussian distribution to view the spread of the data within each group of participants using the same methodology. Gaussian distribution graphs to reflect cumulative accuracy and precision of testing. Gaussian distribution of the Mean (SDI) values gives an indication of overall accuracy, whilst the spread of the data reflects precision i.e. the SD (of the Mean SDI). For the purpose of this analysis, once again the three most commonly used methodologies (BDS FACSCount™, BDS TruCOUNT™ MultiSET™ and SP PLG-CD4) were assessed (Figures 3.17 to 3.21). Partec® was not used for the Gaussian distribution plots as there was not enough data to generate meaningful plots.

3.9.1 Radar plots showing a group of participants using the same methodology

To assess the longitudinal accuracy and precision of a group of laboratories using the same CD4 methodology (BDS FACSCount™, BDS TruCOUNT™ MultiSET™ and SP PLG-CD4 and Partec®), the standardised individual laboratory SDI values were pooled across the first 20 trials

and grouped according to CD4 methodology used. Thus a mean (SDI) was calculated per user group per trial.

Radar plots show the mean of the pooled SDI values of a group of users, plotted trial by trial of a group of participants using the same methodology.

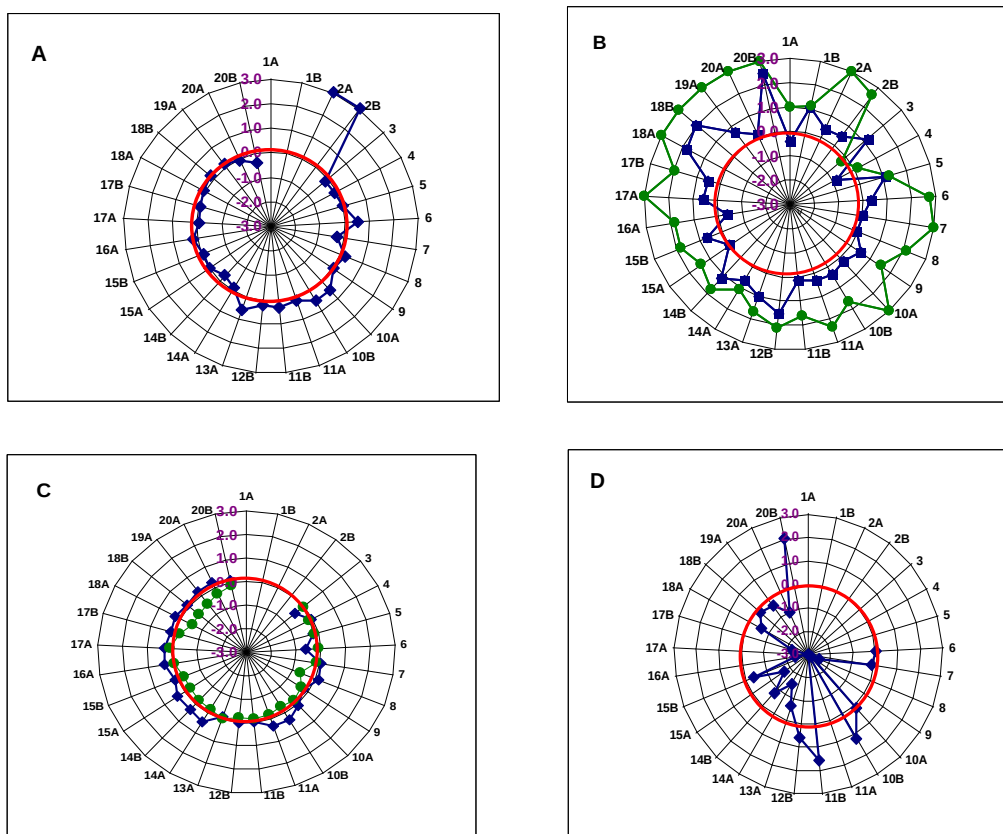


Figure 3.9: Radar plots illustrating the longitudinal performance (SDI) or the 4 most commonly used CD4 methodologies used across the first 20 trials. A= BDS FACSCount™, B= BDS TruCOUNT™ MultiSET™, C= SP PLG-CD4 and D= Partec®) users. SDI values are plotted for both the absolute CD4 count (Blue ♦) and %CD4 of lymphocyte values (Green ●) (BDS FACSCount™ and Partec® systems were not able to generate CD4%Ly).

3.9.2 Overall methodology comparisons

In this pooled SDI analysis grouped according to the same CD4 methodology, the calculated mean of the pooled SDI's, i.e., the mean (SDI) reflects longitudinal accuracy within a group of CD4 users. The SD (SDI) of the mean of the SDI namely, SD (Mean SDI), reflects longitudinal precision of a group of CD4 users. Ideal accuracy mean (SDI) values should be expected to be 0.0 with an

expected range of -1 to +1. Ideal precision SD (Mean SDI) values should be expected to be 1.0 with an expected range of 0.0 to 1.0. Results were plotted as Gaussian distribution graphs for visual interpretation with acceptable ranges indicated and outliers identified as values outside these ranges. On comparing absolute CD4 counts (Figure 3.10), BDS FACSCount™ and SP PLG-CD4 showed similar longitudinal patterns of accuracy and precision. BDS TruCOUNT™ MultiSET™ showed poorer overall accuracy and precision with more variability in results (spread) compared to either BDS FACSCount™ or PLG-CD4 groups.

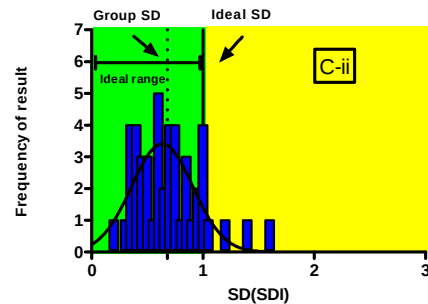
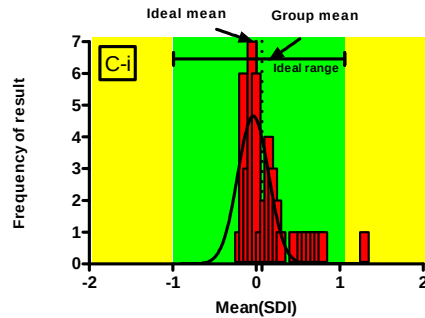
BDS TruCOUNT™ MultiSET™ and SP PLG-CD4 methodologies were compared for CD4%Ly (BDS FACSCount™ technology could not produce a CD4% of lymphocytes at the time of the study). SP PLG-CD4 showed better accuracy and precision than BDS TruCOUNT™ MultiSET™ showed acceptable accuracy, though with a wider spread (variability) of data. Their precision showed that $\pm 50\%$ of the calculated SD of SDI's was >1 (poor performance). Overall precision for PLG-CD4 showed 4 trials with variability (>1) indicating poor performance for these trials (Figure 3.11).

Absolute CD4 Comparison of Methodologies

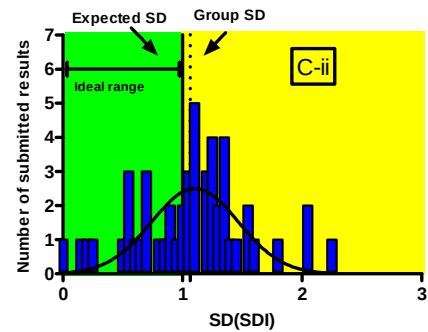
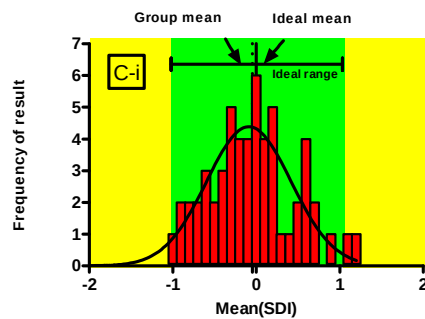
Accuracy

Precision

FACSCCount



TruCount MultiSet



SP PLG/CD4

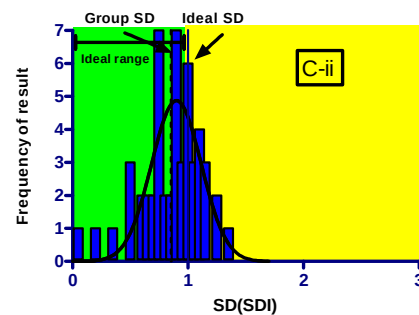
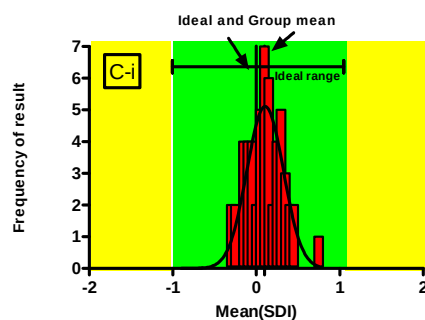


Figure 3.10

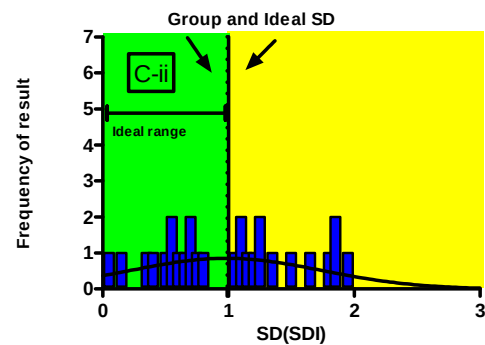
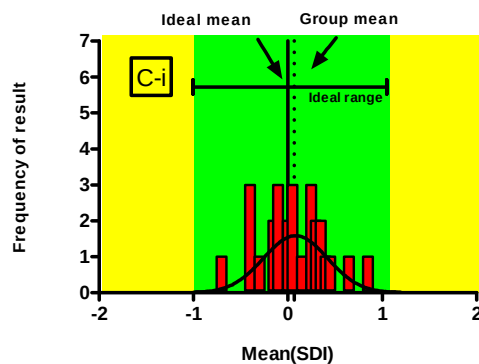
Summary of Gaussian distribution curves showing longitudinal accuracy (mean SDI, C-i) and precision (SD of mean SDI), C-ii for CD4abs counts between technologies. Green areas represent the acceptable limits for both Mean SDI and SD of (mean SDI) values, while yellow areas indicate values outside the acceptable limits. Ideal values for both mean SDI (0) and SD of (mean SDI) (1) are indicated.

CD4% of Lymphocytes Comparison of Methodologies

Accuracy

Precision

TruCount MultiSet



SP PLG/CD4

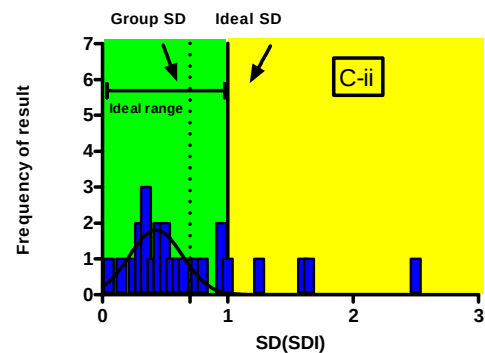
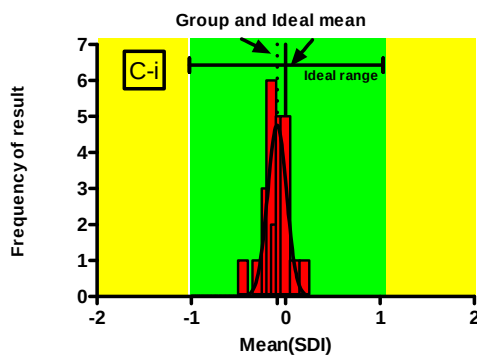


Figure 3.11: Summary of Gaussian distribution curves showing longitudinal accuracy (mean SDI, Ci) and precision (SD of mean SDI), Cii) for CD4%Ly between technologies. Green areas represent the acceptable limits for both Mean SDI and SD of (mean SDI) values, while yellow areas indicate values outside the acceptable limits. Ideal values for both mean SDI (0) and SD of (mean SDI) (1) are indicated.

3.9.3 BDS FACSCount™ methodology

Pooled SDI data reflecting longitudinal accuracy and precision across 20 trials showed that laboratories using BDS FACSCount™ could consistently generate accurate and precise absolute CD4 counts irrespective of whether normal or low value EQA material was used (Figure 3.12).

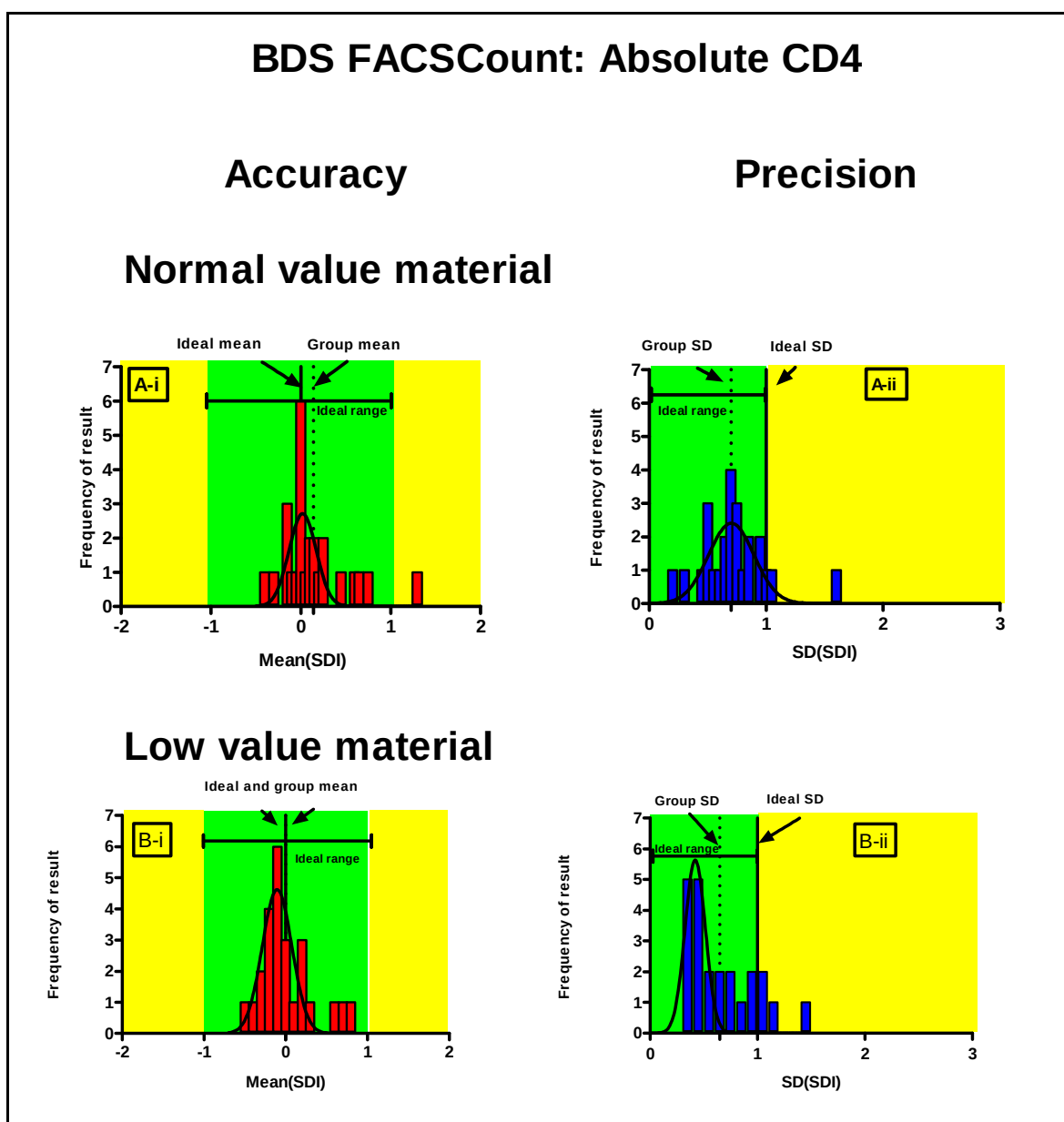


Figure 3.12: Gaussian distribution curves showing longitudinal accuracy (mean SDI, Ai and B i) and precision (SD of mean SDI), Aii and B ii) for BDS FACSCount™ users for CD4 abs counts. Green areas represent the acceptable limits for both Mean SDI and SD of (mean SDI) values, while yellow areas indicate values outside the acceptable limits. Ideal values for both mean SDI (0) and SD of (mean SDI) (1) are indicated.

The distribution curves were tight and majority of results indicated good longitudinal accuracy and precision for absolute CD4 counts (in green area, Figure 3.12 above). Only one trial showed poorer accuracy and precision (Trial 2). This was due to two outliers where one participant reported CD4 abs counts for both EQA samples (Trials 2A and 2B) of 939cells/ μ l (pool mean 604.9 cells/ μ l) and 870cells/ μ l (pool mean 609.3 cells/ μ l) respectively. On low EQA material, the longitudinal follow-up showed good accuracy (mean SDI) but very slight poorer precision than with normal EQA material. This however did not impact on the overall good performance of this methodology on the CD4 AFREQAS scheme.

3.9.4 BDS TruCOUNT™ MultiSET™ CD45 bright gating methodology

Longitudinal accuracy and precision across 20 trials showed that laboratories using TruCOUNT™ MultiSET™ CD45 bright gating methodology, indicated acceptable accuracy (green area Figure 3.13), but demonstrated larger variability (spread of data) for both CD4 abs counts and CD4%Ly values for normal and low EQA samples. Precision between laboratories was not ideal, with 50% of calculated SD of their SDI values, falling in the range of 1-2 (i.e. poorer performance, yellow area, Figure 3.13). Lack of precision of pipetting was the main contributing cause here. Generally, this group of users was not able to reliably generate CD4%Ly values consistently. The between laboratory precision within most trials was poor (largely contributed by the inability to manually gate CD45+ bright populations).

However, although the overall performance of this technology was poorer than the BDS FACSCount™ user group, the individual performance of some laboratories using this system was nevertheless excellent (see Appendix D) (104).

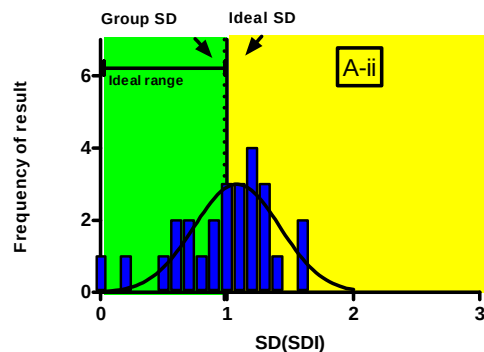
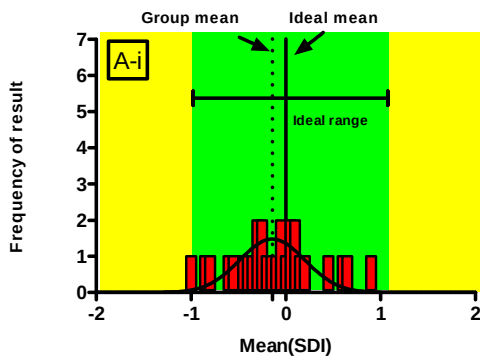
Further, in some trials, the group performance was also better (see Figure 3.9 B, trials 6-11B when reporting CD4 abs counts and trials 13A-16A en reporting CD4%Ly values).

TruCount MultiSet: Absolute CD4

Accuracy

Precision

Normal value material



Low value material

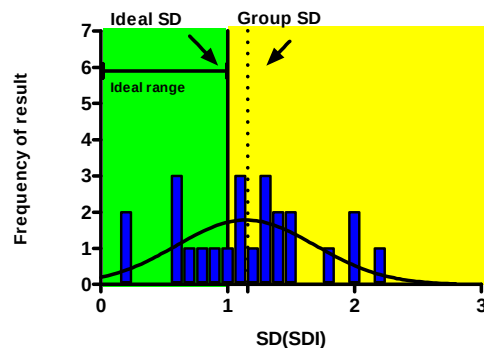
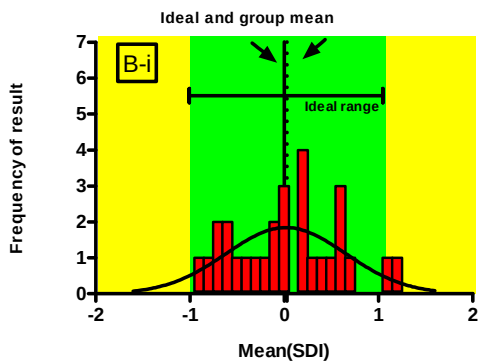


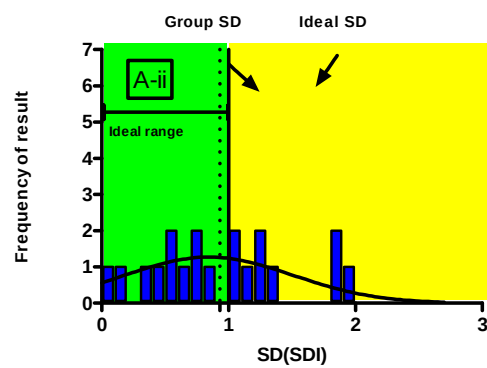
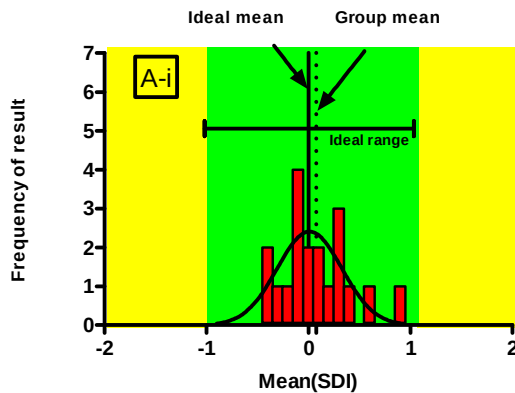
Figure 3.13: Gaussian distribution curves showing longitudinal accuracy (mean SDI, Ai and B i) and precision (SD of mean SDI, Aii and B ii) for TruCOUNT™ MultiSET™ CD45 bright gating users CD4 abs counts. Green areas represent the acceptable limits for both Mean SDI and SD of (mean SDI) values, while yellow areas indicate values outside the acceptable limits. Ideal values for both mean SDI (0) and SD of (mean SDI) (1) are indicated.

TruCount MultiSet: CD4 % of Lymphocytes

Accuracy

Precision

Normal value material



Low value material

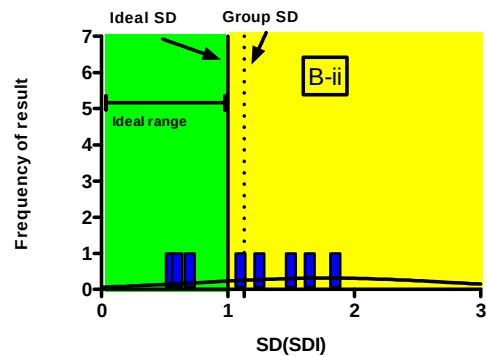
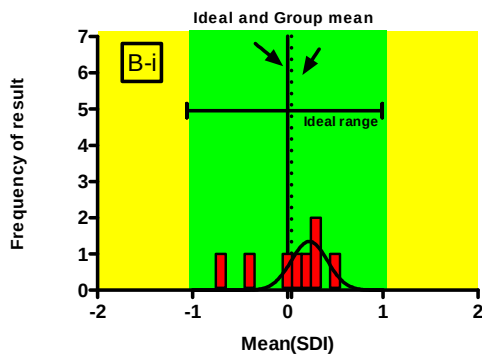


Figure 3.14: Gaussian distribution curves showing longitudinal accuracy (mean SDI, Ai and B i) and precision (SD of mean SDI), Aii and B ii) for TruCOUNT™ MultiSET™ CD45 bright gating users for CD4%Ly. Green areas represent the acceptable limits for both Mean SDI and SD of (mean SDI) values, while yellow areas indicate values outside the acceptable limits. Ideal values for both mean SDI (0) and SD of (mean SDI) (1) are indicated.

3.9.5 SP PLG-CD4 methodology

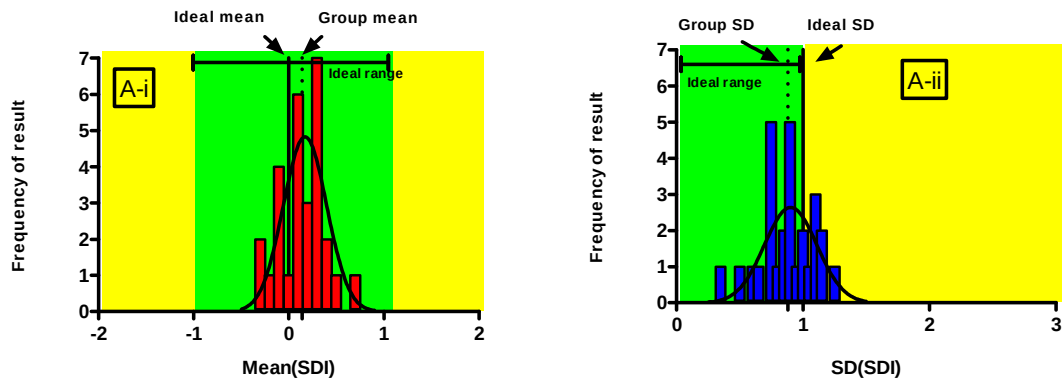
Longitudinal accuracy and precision analyses across 20 trials showed that laboratories using SP PLG-CD4 could reliably generate accurate and precise CD4 abs counts and CD4%Ly values for both normal and low value EQA material. The distribution curves (Figure 3.15) were tight and good longitudinal accuracy shown for absolute CD4 counts for normal and low EQA samples analysed. Overall precision of the PLG-CD4 group was acceptable for the CD4%Ly (with majority of trials Mean SDI <1) (Figure 3.16), although there was more variability (spread of data) on the low EQA material compared to normal data.

PLG/CD4: Absolute CD4 count

Accuracy

Precision

Normal value material



Low value material

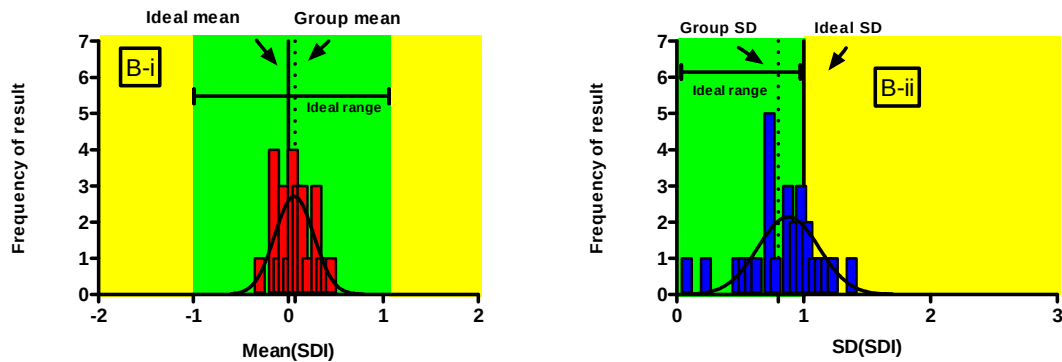


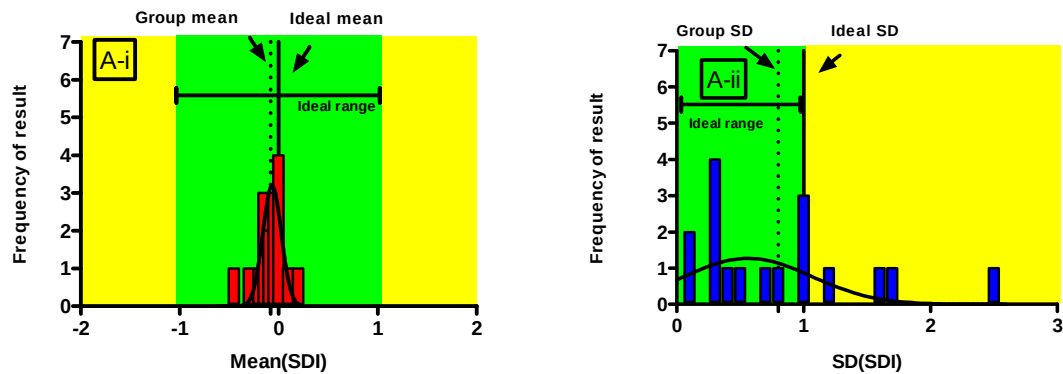
Figure 3.15: Gaussian distribution curves showing longitudinal accuracy (mean SDI, Ai and B i) and precision (SD of mean SDI), Aii and B ii) for SP PLG-CD4 users CD4 abs counts. Green areas represent the acceptable limits for both Mean SDI and SD of (mean SDI) values, while yellow areas indicate values outside the acceptable limits. Ideal values for both mean SDI (0) and SD of (mean SDI) (1) are indicated.

PLG/CD4:CD4 % of lymphocytes

Accuracy

Precision

Normal value material



Low value material

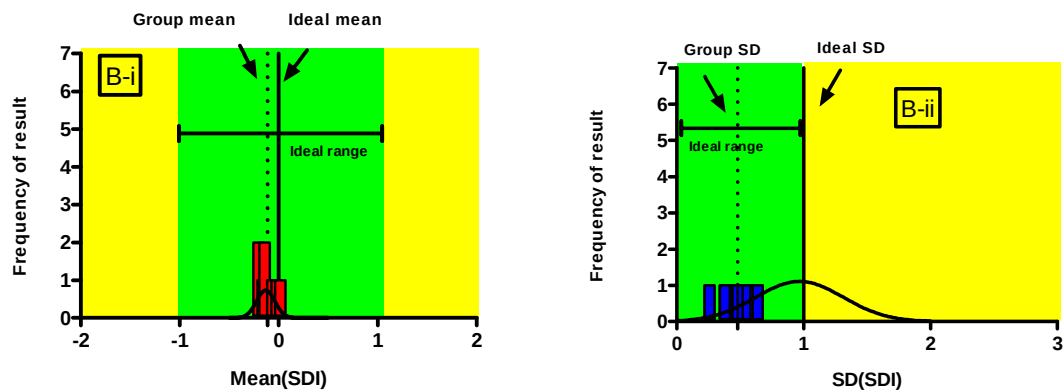


Figure 3.16: Gaussian distribution curves showing longitudinal accuracy (mean SDI, Ai and B i) and precision (SD of mean SDI), Aii and B ii) for SP PLG-CD4 gating users for CD4%Ly. Green areas represent the acceptable limits for both Mean SDI and SD of (mean SDI) values, while yellow areas indicate values outside the acceptable limits. Ideal values for both mean SDI (0) and SD of (mean SDI) (1) are indicated.

3.10 Within trial precision of Southern African laboratory networks.

Five countries with a National programme for CD4 testing participated on the CD4 AFREQAS scheme. These included South Africa National Health Laboratory Service (SA-NHLS), Botswana National Government Laboratories, Namibia National Institute of Pathology (NIP), Lesotho National Government Laboratories and Zimbabwe National Government Laboratories. SA-NHLS enrolled their National laboratories using SP PLG-CD4 on the program from Trial 6 in 2004. Botswana enrolled one site on the scheme from Trial 3 in 2003 and later enrolled eleven laboratories from Trial 15 in 2005 onwards. Namibia enrolled one site on the scheme from Trial 6 in 2004 and a further four of their laboratories from Trial 10 in 2005. Lesotho enrolled eleven laboratories from Trial 14 in 2005 and Zimbabwe enrolled one site from Trial 17 in 2006 and eighteen laboratories from Trial 19 in 2006 onwards (Table 3.8).

Table 3.8: The precision between laboratories on the same national program is reflected as the %CV of the submitted data (Figures 3.17-3.18).

	Methodology used	Total number of participating laboratories	Total number of laboratories using described methodology	Percentage laboratories using described methodology
<u>South Africa</u> National Health Laboratory Service	Single platform Panleucogated CD4 (SP PLG-CD4)	40	40	100
<u>Botswana</u> National Government Laboratories	BDS TruCOUNT™ MultiSET™ CD45 bright gating	12	4	33
	BDS FACSCount™		6	50
	Unknown		2	17
<u>Namibia</u> National Institute of Pathology	Single platform Panleucogated CD4 (SP PLG-CD4)	5	5	100
<u>Lesotho</u> National Government Laboratories	BDS TruCOUNT™ MultiSET™ CD45 bright gating	11	3	27
	Partec®		8	73
<u>Zimbabwe</u> National Government Laboratories	BDS TruCOUNT™ MultiSET™ CD45 bright gating	19	1	5
	BDS FACSCount™		4	21
	Partec®		4	21
	Dual platform MultiSET™ Primary CD3 gating		5	26
	BDS TruCOUNT™ MultiSET™ Primary CD3 gating		1	5
	Unknown		4	21

3.10.1 Overall performance of Southern African laboratory networks

South African NHLS laboratories showed the most consistent performance across their course of participation on the CD4 AFREQAS scheme, with a mean %CV of 7.99 ± 2.6 for CD4 abs counts and a mean %CV of 4.8 ± 2.4 for the CD4%Ly. No outliers were detected for this group. The Namibian laboratories also showed consistency in results, with results from one trial (Trial 17B) removed as an outlier (%CV of 49.7%). For Trial 17B, only three participants submitted results with one result recorded as an outlier. The high %CV was due to a single outlier where one participant reported a CD4 abs count of 183 cells/ μ l (pool mean 141.7 cells/ μ l). Overall they showed a mean %CV of $12 \pm 11.7\%$ for CD4 abs counts and 4.6 ± 2.8 for CD4%Ly. Botswana laboratories showed acceptable (with trial 15B as outlier removed) precision with a trimmed mean %CV of 10.9 ± 8.7 for CD4 abs counts and 8.45 ± 4.36 for CD4%Ly. However, the inconsistency of performance was reflected in the increased SD of 8.7 (spread of data) compared to SD of 2.6 and 5.5 for South Africa and Namibia respectively (Figures 3.17-3.18 and Table 3.9).

Lesotho laboratories had mean %CV values of $31 \pm 11\%$ for CD4 abs counts, indicating poor precision between laboratories of this network. Similar performance in the reporting of CD4%Ly was reflected by the mean %CV of 9.4 ± 8.5 , indicating inconsistency (poor precision) over their period of participation on the CD4 AFREQAS scheme.

Zimbabwean laboratories had mean %CV values of 38%. On removing three outliers (Trials 9, 13A and 20B), the mean %CV for CD4 abs counts decreased to $14.9 \pm 12.8\%$, still indicating a wide spread of data and inconsistency of precision. Their %CV for CD4%Ly was 15.9 ± 19.2 , again indicating poor ongoing precision. In a sub analysis of the BDS FACSCount™ user group (4 participants), this group had a %CV (<8%) comparable to the reported BDS FACSCount™ user group (Figure 3.6).

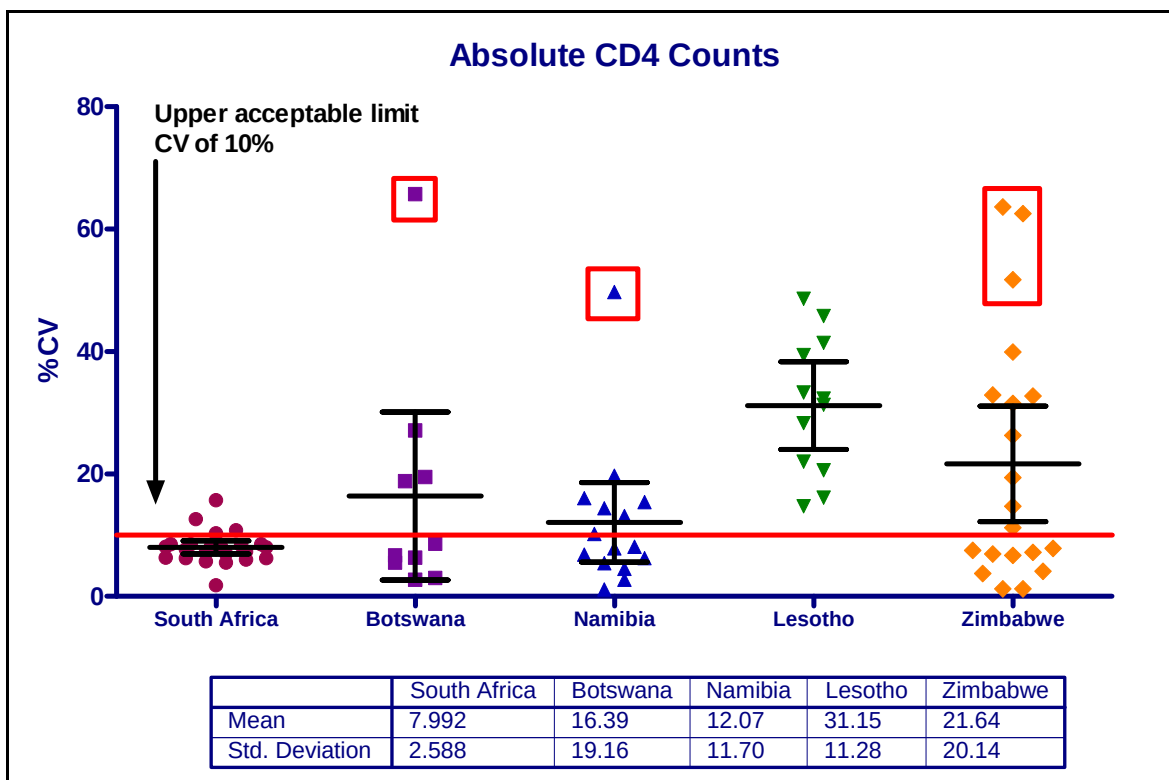


Figure 3.17: Between laboratory precision (%CV) of network laboratories reporting absolute CD4 counts

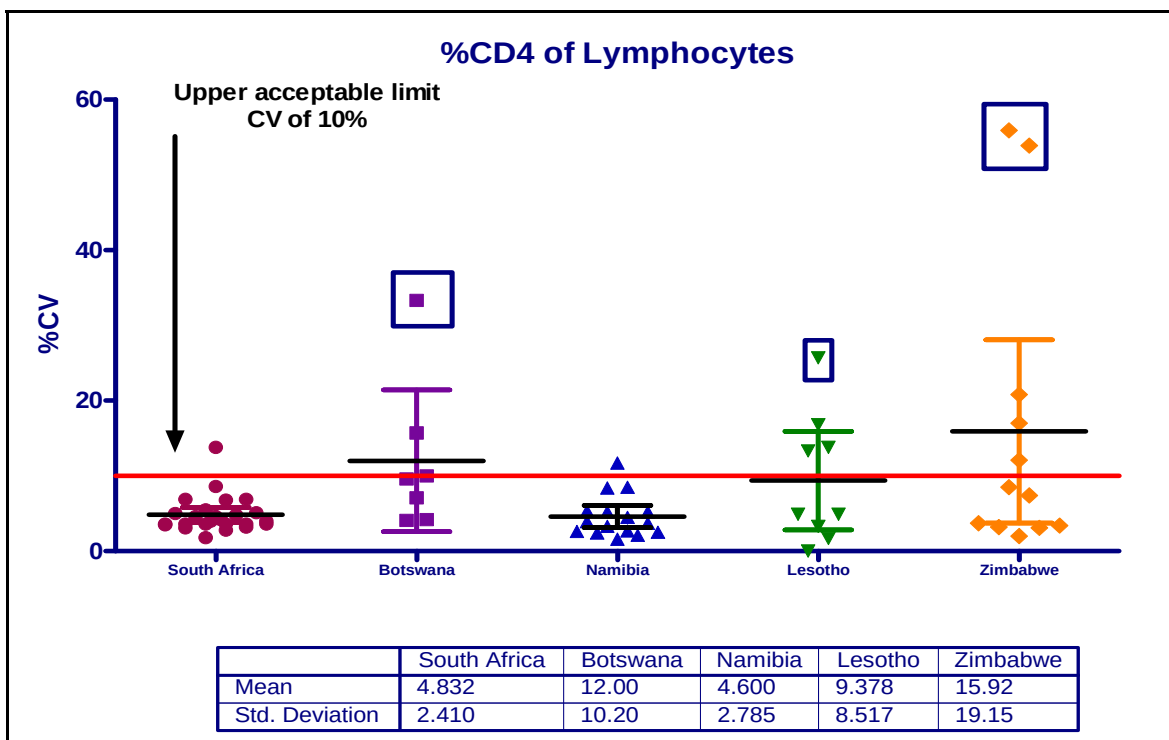


Figure 3.18: Between laboratory precision (%CV) of network laboratories reporting CD4 % lymphocytes

Table 3.9: Comparison of statistics for laboratory precision (%CV) of Southern African Network laboratories for CD4 abs counts and CD4%Ly, with outliers and outliers removed.

Absolute CD4 counts	South Africa	Botswana	Namibia	Lesotho	Zimbabwe
All data	25.0	10.0	15.0	12.0	20.0
Mean	8.0	16.4	12.1	31.2	21.6
Std. Deviation	2.6	19.2	11.7	11.3	20.1
Outliers removed		1.0	1.0		3.0
Mean		10.9	9.4		15.0
Std. Deviation		8.7	5.5		12.8
CD4% of Lymphocytes	South Africa	Botswana	Namibia	Lesotho	Zimbabwe
All data	25.0	7.0	16.0	9.0	12.0
Mean	4.8	12.0	4.6	9.4	15.9
Std. Deviation	2.4	10.2	2.8	8.5	19.2
Outliers removed		1.0		1.0	2.0
Mean		8.5		7.3	8.1
Std. Deviation		4.4		6.3	6.5

3.10.2 Analysis of performance between Southern African laboratory networks for normal versus low EQA material

As shown in the graphs below (Figure 3.19), the SA-NHLS laboratories showed consistent precision for CD4 abs counts and CD4%Ly using the SP PLG-CD4 method regardless of the level of EQA material analysed. Botswana only showed better overall precision on low EQA material for CD4%Ly. Namibia showed poor precision on low EQA material for CD4 abs counts. Lesotho showed better overall precision on normal EQA material for CD4%Ly. Zimbabwe showed poorer precision for CD4 abs counts and CD4%Ly regardless of the level of EQA material analysed (Figure 3.19 and Table 3.10).

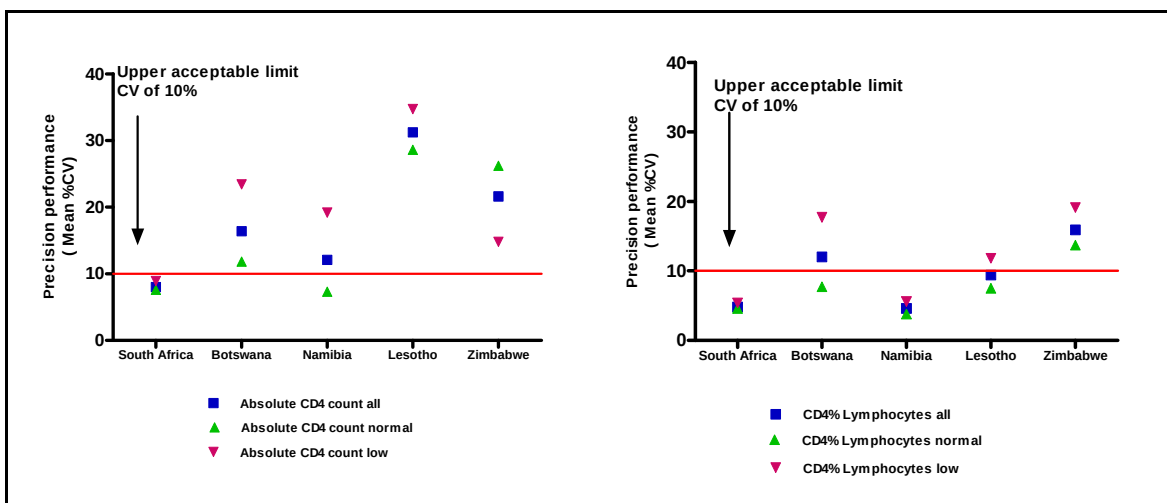


Figure 3.19: Mean %CV reflecting precision between Southern African laboratory networks for all shipments grouped according to normal and low EQA material analysed.

Table 3.10: Summary of normal versus low EQA material precision for Southern African Laboratory networks.

<u>CD4 abs counts</u>	<u>South Africa</u>		<u>Botswana</u>		<u>Namibia</u>		<u>Lesotho</u>		<u>Zimbabwe</u>	
	Normal	Low	Normal	Low	Normal	Low	Normal	Low	Normal	Low
Mean	7.6	8.9	11.8	23.4	7.3	19.2	28.6	34.7	26.2	14.8
<u>CD4%Ly</u>	<u>South Africa</u>		<u>Botswana</u>		<u>Namibia</u>		<u>Lesotho</u>		<u>Zimbabwe</u>	
	Normal	Low	Normal	Low	Normal	Low	Normal	Low	Normal	Low
Mean	4.6	5.4	7.7	17.7	3.8	5.6	7.5	11.8	13.7	19.1

3.10.3 Longitudinal Analysis of individual National networks

Representative examples of the national networks' (shown in Figures 3.20-3.24) longitudinal SDI follow-up performance assessments over the twenty trials were graphically represented to show accuracy and precision. Radar (circular) plots of SDI values were used to assess ongoing performance of individual laboratories, within a network to help identify problems.

3.10.3.1 South African National Health Laboratory Service (SA-NHLS)

The SA-NHLS laboratories showed consistent good performance across all the trials they participated in (from trial 3 onward). A representative selection of longitudinal performance for this group is shown in Figure 3.20. Occasional outliers (examples A, B, C, E and F) and one non-submission of results (example F) are indicated in the examples below. The overall performance of the group was however not adversely affected by isolated laboratory problems (Figures 3.17-3.18).

Examples of laboratories that reported the CD4% of WBC instead of the correct CD4%Ly on one trial, is shown in Figure 3.20 A and B. Laboratory B and H, had incorrect gating by cutting off the CD4 population on the CD4/SS plot and submission of incorrect CD4 abs counts, on one trial. An example of a laboratory (C) that had an outlying result for CD4 abs counts and CD4%Ly on Trial 6 and to ensure correct CD4 abs counting, with further corrective action feedback this participant improved on their pipetting skills (See section 3.11, Investigation of laboratories with outlying results, Figure 3.28). Examples of laboratories that showed consistently good performance across all trials is shown in Figure 3.20 D and G. Majority of the laboratories on the NHLS network performed very well. This good within network performance is largely attributed to the fact that the South African NHLS standardised their methodology across all laboratories to the SP PLG-CD4 method (84). SA-NHLS further applied a proactive approach to training by maintaining quality between laboratories through the implementation of simplified PLG- CD4 testing, as well as through a novel internal quality control system using bead count rates with SP PLG-CD4 method (84, 98) and participation on the CD4 AFREQAS scheme (21). These findings are confirmed on the within trial precision analysis (Radar mean (SDI) plots Figure 3.9 and Gaussian distribution plots Figures 3.10-3.11 and 3.15-3.16).

All SP PLG-CD4

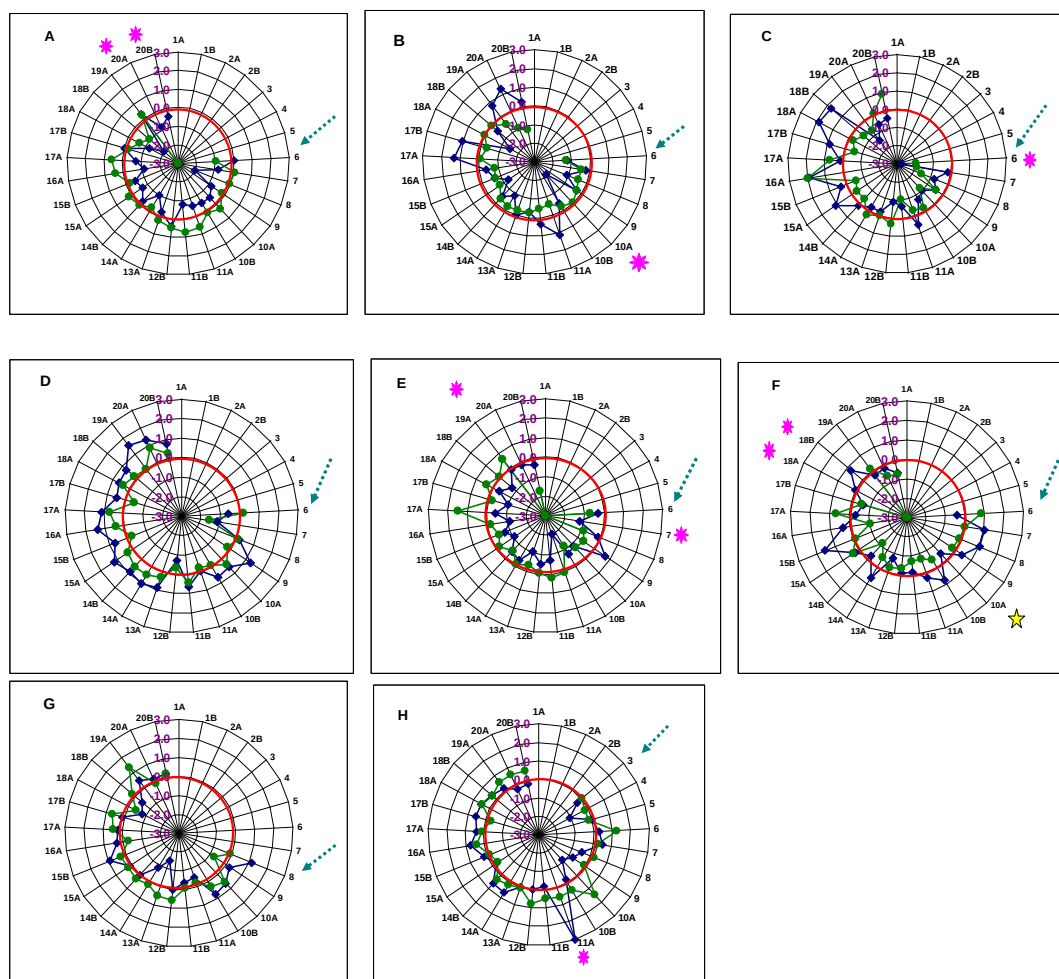


Figure 3.20: South African (National Health Laboratory Service National Programme). SDI values obtained from representative SA-NHLS laboratories (A-H) using SP PLG-CD4. The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green ●) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. * Represents outliers, while ☆ represents non-submission for a particular trial

3.10.3.2 The Botswana National Government Laboratory network

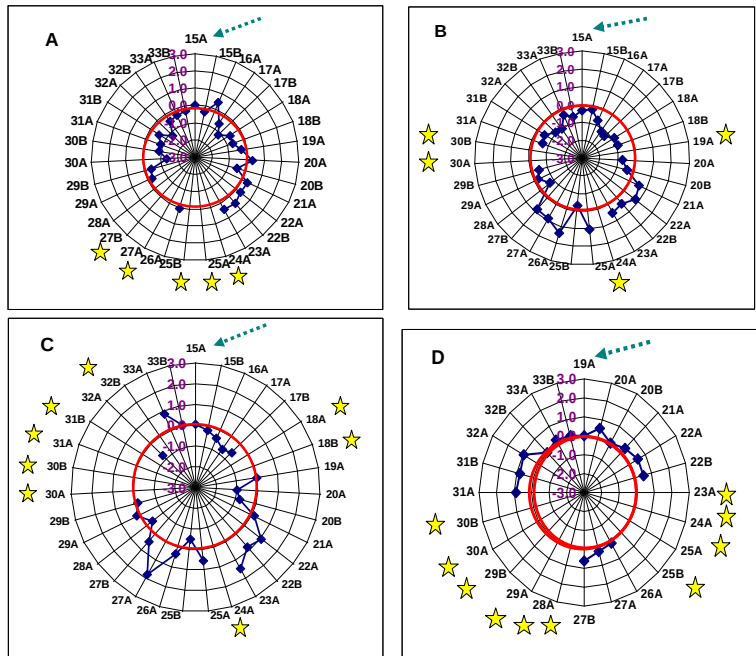
In January 2002, Botswana introduced their ARV roll-out program. Initially, one laboratory joined the CD4 AFREQAS scheme in June 2003, with 8 more laboratories joining in November 2005. By July 2006 there were a total of twelve laboratories from Botswana participating.

The Botswana National Government Laboratory Programme did not use a standardised method for CD4 testing or implement a national training programme (Table 3.8). Majority of laboratories used the BDS FACSCount™ (n=6) which gave precise and accurate results. The remaining laboratories used the BDS TruCOUNT™ MultiSET™ system according to the supplier's recommendations. Despite this, performance was inconsistent and generally poor. Two participants that did not state which technology they used. However, the BDS FACSCount™ user group within this Botswana network showed reliable and reproducible performance (Figure 3.21). This is shown on the radar SDI performance of longitudinal representative participants of the Botswana group (Figure 3.21 A-D).

Botswana joined the scheme from Trial 15 and to make the selection for example more representative, Trials 21 to Trials 33 were include. In Figure 3.21 examples of laboratories performance are shown. These laboratories (A and B) using BDS FACSCount™ showed consistently good performance across trials. The non submissions (A-D) had problems with procurement and down time of the BDS FACSCount™. Laboratory E, F, G and H generated outlying results with SDI values of >3.0 as well as non submission of results. In communication with Laboratory E and H, it transpired that they had experienced problems in getting additional training on their instrument and software applications.. Subsequently, these sites received additional training from BDS on their instrument and software applications. Since Trial 20 their performance has improved dramatically reflecting the impact of appropriate interventions. Some laboratories showed no improvement despite corrective action advice.

In consultation with Laboratory F and G it was revealed they had their FACSCalibur serviced and the staff received technical training. However, these two sites did not improve on their performance on subsequent trials.

BDS FACSCCount™ users A-D



BDS TruCOUNT™ CD45 bright gating users E-H

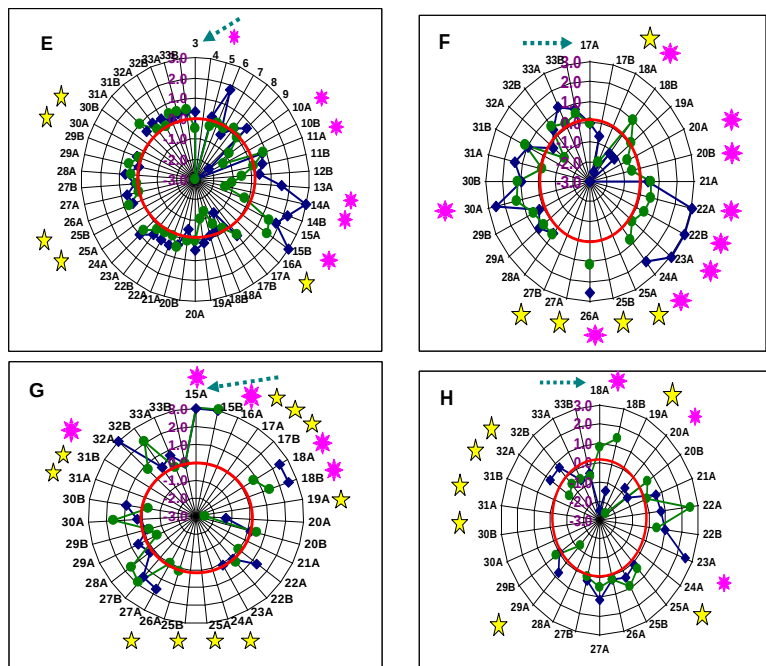


Figure 3.21: Botswana National Network laboratories (A-H).
The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green •) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. *Represents outliers, while★ represents non-submission for a particular trial

3.10.3.3 The Namibia National Institute of Pathology (NIP)

The Namibia NIP had standardised the PLG-CD4 methodology across five national health laboratories. These laboratories showed overall reliable and reproducible results, following implementation and rollout of their national anti retroviral treatment programme. Analysis of their performance requires bead rate monitoring. Introduction of bead count rates (4, 5, 6) was recommended to monitor poor pipetting techniques (imprecision and inaccuracy) and improve within sample internal quality control. Initially, the participating laboratories had received intensive training from Beckman Coulter, South Africa and a follow-up CD4 PLG workshop from the NHLS CD4 Reference laboratory but there was no formal NIP training programme. In Figure 3.22 (A-E), examples are shown of laboratories within this network. These laboratories showed consistently good precision on CD4%Ly. However, the precision was more erratic on CD4 abs counts.

All SP PLG-CD4

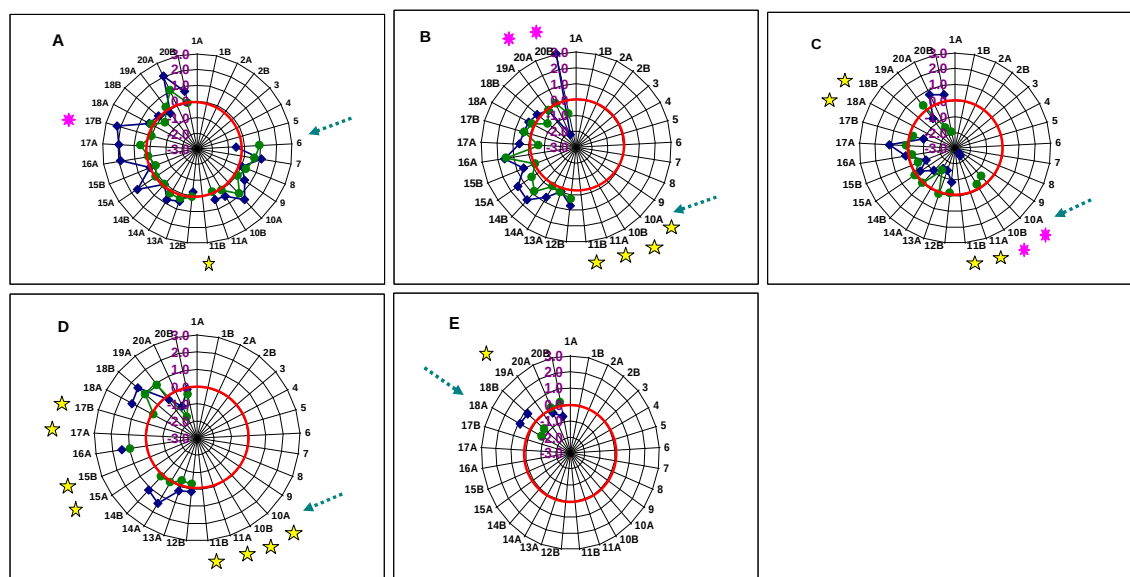


Figure 3.22: Namibia National Institute of Pathology laboratories (A-E).

The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green ●) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. *Represents outliers, while ☆ represents non-submission for a particular trial

3.10.3.4 The Lesotho National Government Laboratory network

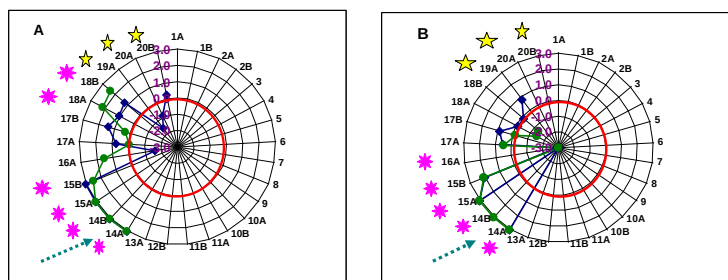
In 2005, Medecins Sans Frontiers (Doctors without borders), the Ministry of Health and Social Welfare and the Global fund, launched a joint programme in Lesotho to provide antiretroviral treatment at the primary health care level to HIV/AIDS patients. In October 2005, eleven laboratories enrolled on the CD4 AFREQAS scheme. The Lesotho National programme did not standardise (Table 3.8) on a CD4 methodology across the laboratories. BDS TruCOUNT™ MultiSET™ incorporating CD45 bright gating was used in three of the eleven laboratories while the remaining 8 sites used Partec® instruments.

The poorer performance of the Lesotho national network is similar to other networks using non-standardised testing. Across the trials the poor performance is reflected by a wide variation of results submitted. The performance of laboratories using BDS TruCOUNT™ MultiSET™ with bright gating showed a wide variation of submitted results and this is shown on the graphical radar SDI performance of representative participants (Figure 3.23 A-B). Contributing problems included poor understanding and application of the automated algorithm software especially related to gating and manual identification of relevant populations, poor pipetting resulting in inaccurate volumes of blood being pipetted and calibration of pipettes not monitored. The performance of the Partec ® users was especially poor. Although more than 75% of laboratories used one methodology, the impact of standardising methodology has been lost due to poor manufacturer support and instrument maintenance. Training, to ensure consistency of accurate and precise reporting was lacking for pipetting, calibration of pipettes, no basic training on maintenance and repair to improve the down time of their Partec ® instruments.

Examples of BDS TruCOUNT™ MultiSET™ incorporating CD45 bright gating user group are shown in Figure 3.23 A and B. These participants joined the CD4 AFREQAS scheme from Trial 14 and from this trial to Trial 20 demonstrated poor performance with respect to both CD4 abs counts and CD4%Ly results. This is reflected by the high number of outlying results (>3.0) and non submission of results. These two participants did not respond to suggested corrective action

interventions. Examples of the Partec® user group are shown in Figure 3.23 C-H. These participants demonstrated poor performance with respect to CD4 abs counts. This is reflected by the high number of outlying results (>3.0) and non submission of results. These sites also did not respond to suggested corrective action interventions.

BDS TruCOUNT™ CD45 bright gating users A-B



Partec™ users C-H

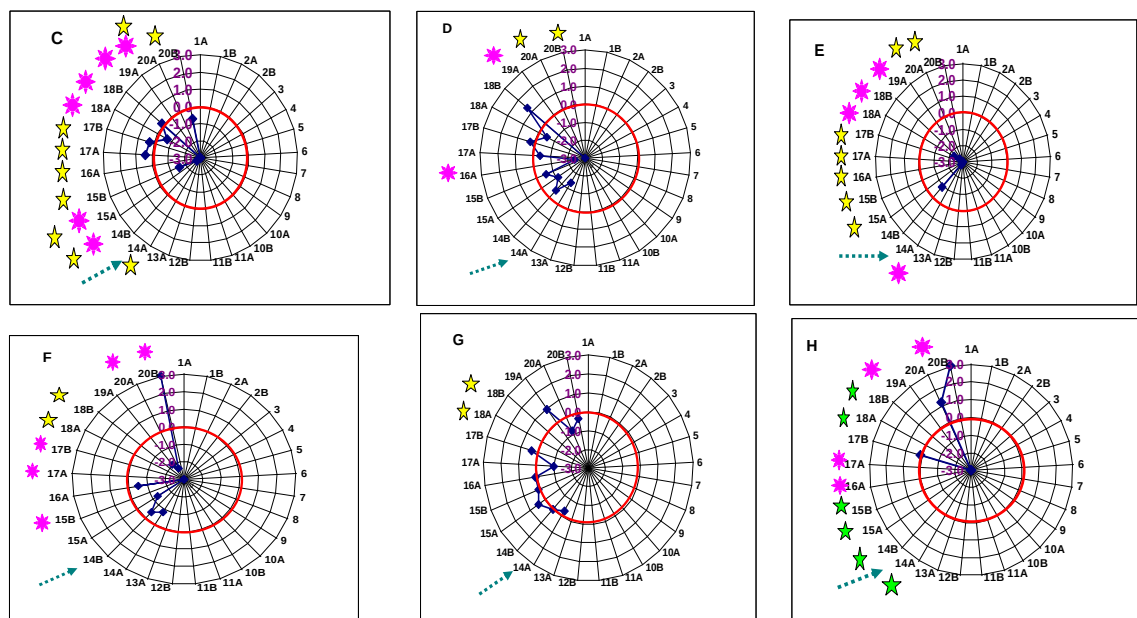


Figure 3.23: Lesotho National Government laboratories (A-H). The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green ●) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. *Represents outliers, while ☆ represents non-submission for a particular trial

3.10.3.5 The Zimbabwe National Government Laboratories

In March 2006 (Trial 19), one of Zimbabwe's largest referral hospitals joined the CD4 AFREQAS scheme. In July that year, eighteen national district hospital laboratories also joined the programme. No standardised CD4 methodology is used in this network (Table 3.8), with laboratories using either BDS TruCOUNT™/MultiSET™ with bright gating, Primary CD3 gating, dual platform Primary CD3 gating, Partec® or BDS FACSCount™ methodology.

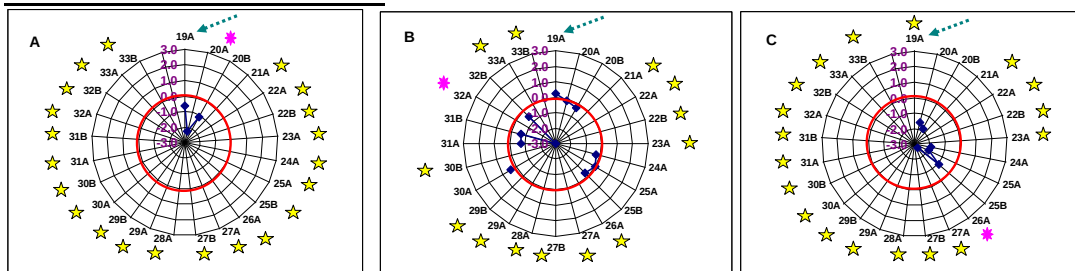
Zimbabwe joined the program from trial 19 and to make the selection for example more representative, Trials 21 to Trials 33 were include. In Figure 3.24 examples of laboratories performance are shown.

The poorer performance of the laboratories as a group comprising the Zimbabwean national network is similar to other networks using non-standardised testing. Here poorer precision and accuracy is reflected not only by the non standardised testing but also in poor understanding of the use of systems used most notably both the BDS TruCOUNT™ MultiSET™ and Partec® volumetric base methodologies. The performance of laboratories using BDS TruCOUNT™ MultiSET™ system showed a wide variation of submitted results (Figure 3.24 D-F). Once again, as had occurred in other sites and networks monitored in this study, the laboratories demonstrated poorer precision and accuracy. Contributing problems included poor understanding and application of the automated algorithm software especially related to gating and manual identification of relevant populations, poor pipetting resulting in inaccurate volumes of blood being pipetted, and calibration of pipettes not being monitored. The performance of the Partec instrument® participants also showed poor performance in spite of perceived national monitoring in their local Zimbabwe National Quality Assessment Programme (Figure 3.24 G-H). Contributing problems included poor pipetting, calibration of pipettes not monitored, no training on maintenance and repair of instruments to improve the down time of their Partec ® instruments and erratic supply of reagents and expired reagents. For improved performance in Zimbabwe, CD4 AFREQAS has recommended standardising on technology and securing improved and perhaps additional supplier support into the country.

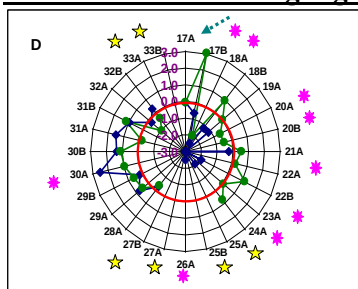
Of note however, and in stark contrast is the excellent performance of those four sites in the Zimbabwe network using the BDS FACSCount™ CD4 system. Here the mean precision (%CV) across these three sites, across relevant trials of participation averaged at <8% seen elsewhere in other programmes (Within trial precision analysis (Radar mean (SDI) plots Figure 3.9 and Gaussian distribution plots Figures 3.10 and 3.12)

The results submitted by BDS FACSCount™ users namely, Laboratories A, B and C were well within the 2 SD limits. However across all trials there was a high rate of non submission of results due to down time of the instruments. The poor performance of the TC/MS system group of users, Laboratories D, E and F is reflected by the high number of outlying results (>3.0) and non submission of results. Laboratory F enrolled on CD4 AFREQAS at Trial 19 and submitted only one result across all subsequent trials. The performance of the Partec ® users Laboratory G and H was especially consistently poor. There was a high non submission rate and this was due to down time of their Partec ® instruments. The performance in particular of the Partec ® users also reflects the importance of manufacturer support for instrument maintenance.

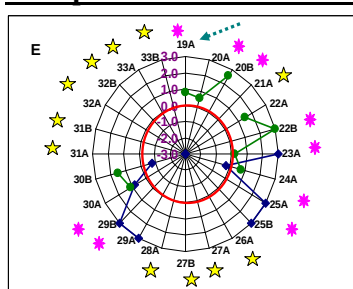
BDS FACSCCount™ users A-C



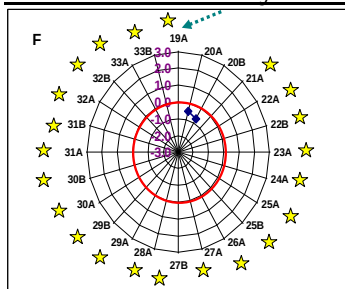
BDS TC/MS CD45 bright gating, D



Dual platform BDS TC/MS CD45 bright gating, E



BDS TC/MS Primary CD3 gating, F



Partec® G-H

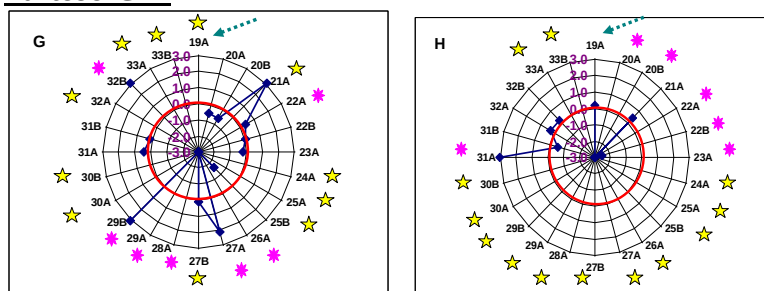


Figure 3.24: Zimbabwean National Government Laboratories National Program. The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green ●) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. ★ Represents outliers, while ☆ represents non-submission for a particular trial

3.10.3.6 Performance of grant supported networks

In addition to the five National programmes there were two networks of laboratories which were grant supported. Significant training of personnel in laboratory management, laboratory methodology and principles of good laboratory practice were conducted in all the sites. Personnel were also given training in South Africa at the National Health Laboratory Service and Witwatersrand based Contract Laboratory Service as well through various commercial suppliers. In contrast to other African networks (with the exception of SA-NHLS), these networks performed better.

The performance of these network groups is reflected in the precision (trimmed %CV) across twenty trials (Figure 3.25). Selective examples of the performance within these groups of laboratories are shown in (Figures 3.26-3.27). A summary of methodologies used in these grant based networks can be seen in Table 3.12.

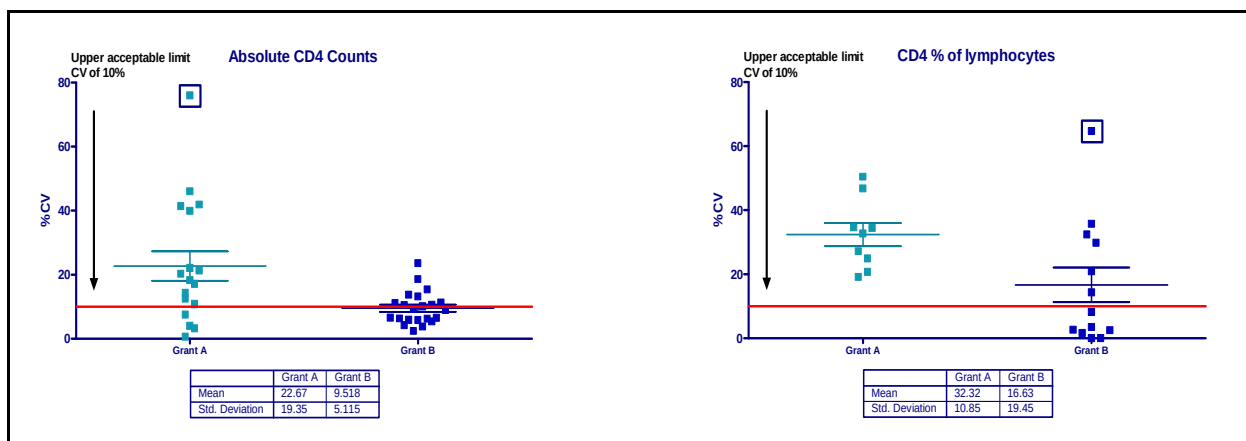


Figure 3.25: Between laboratory precision (%CV) of 2 grant network laboratory groups.

Table 3.11: Summary of methodologies used in grant supported networks

	Methodology used	Total number of participating laboratories	Total number of laboratories using described methodology	Percentage laboratories using described methodology
Grant support Network group “A”	BDS FACSCount™	9	7	78
	BDS TruCOUNT™ MultiSET™		1	11
	CD45 bright gating			
	BDS TruCOUNT™ MultiSET™		1	11
Grant support Network group “B”	Primary CD3 gating			
	BDS TruCOUNT™ MultiSET™	9	2	22
	CD45 bright gating			
	BDS FACSCount™		7	78

Overall across both networks, fourteen (14/18) used BDS FACSCount™ and 4 used BDS TruCOUNT™ MultiSET™ methodology. The performance of the grant “A” network was worse (%CV of 22.7% and 32.4% for CD4 abs counts and CD4%Ly respectively), compared to grant “B” (%CV of 7.8% and 16.6% for CD4 abs counts and CD4%Ly respectively) (Figure 3.25 and Table 3.12).

Table 3.12: Comparison of performance of all and normal versus low EQA material precision for Grant supported networks.

CD4 abs counts	Grant A network		Grant B network	
	Normal	Low	Normal	Low
All mean %CV	22.7		7.8	
Mean %CV	12.7	35.1	7.8	12.6
CD4%Ly	Grant A network		Grant B network	
	Normal	Low	Normal	Low
All mean %CV	32.4		16.6	
Mean %CV	33.4	30.1	11.7	24.1

Within the Grant supported network groups, in general the low EQA material presented higher variability in results, as reflected in poorer precision (%CV) (Table 3.12).

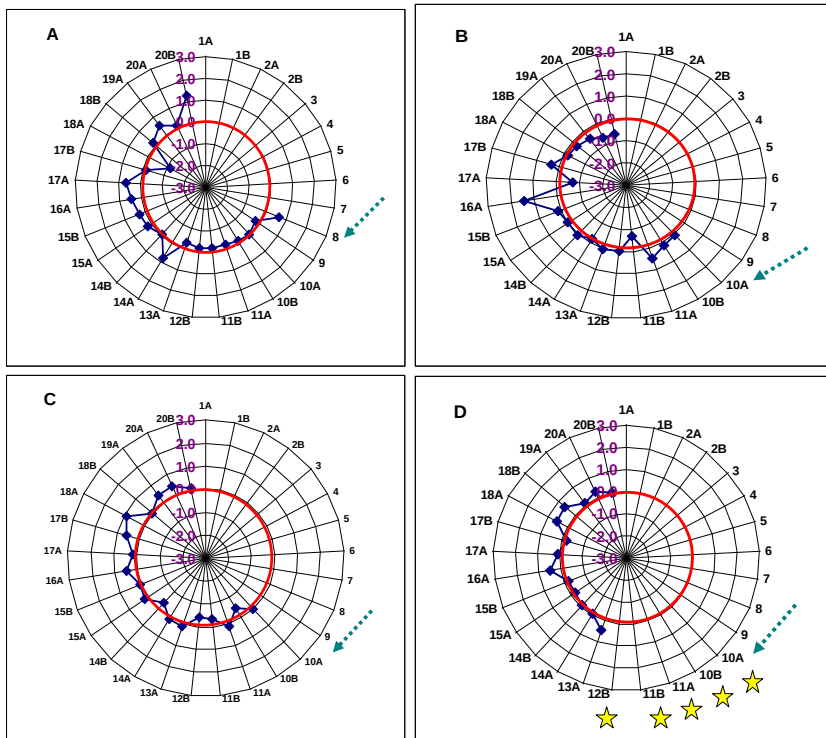
3.10.3.6.1 Grant supported laboratory network A

From August 2003, grant supported laboratory network A was distributed across four countries including Kenya, Rwanda, Uganda and Zambia. The methodologies used by this group consisted of BDS TruCOUNT™ MultiSET™, one group using CD45 bright gating and other using Primary

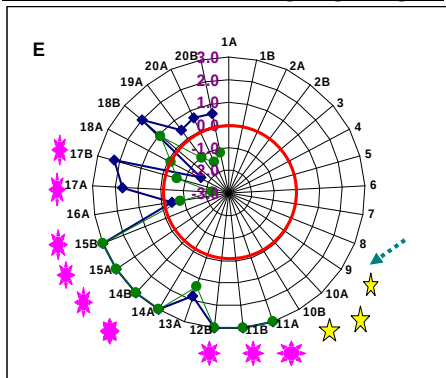
CD3 gating to identify CD4 T cells and a third group using BDS FACSCount™ methodology (Table 3.12).

The subgroup of laboratories within his network using BDS FACSCount™ demonstrated good overall performance for all trials (Figure 3.26 A-D). In contrast, the performance of the BDS TruCOUNT™ MultiSET™ users was poor as reflected by the higher number of outlying results (>3.0) (Figure 3.26 E-F). One of the BDS TC/MS participants (E) had numerous problems with this methodology and after corrective action interventions including an investigation into the multiple outlying results (refer to 3.11.2) this participant generated good results from Trial 18 and all subsequent trials.

BDS FACSCCount™ users A-D



BDS TC/MS CD45 bright gating, E



BDS TC/MS Primary CD3 gating, F

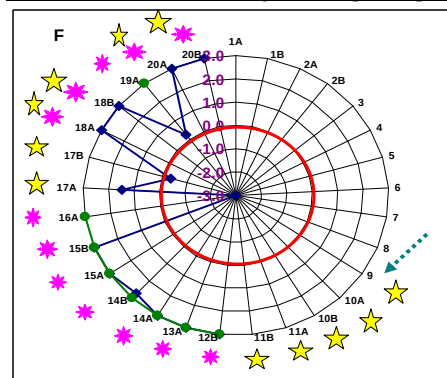


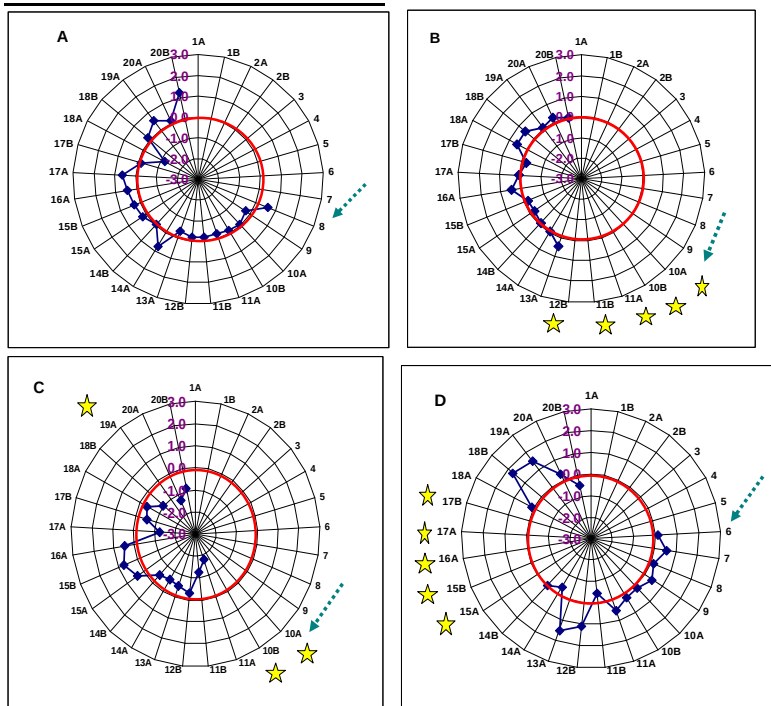
Figure 3.26: Grant supported network “A” Africa Programme. SDI values obtained from representative African laboratories.
The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green ●) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. *Represents outliers, while ★ represents non-submission for a particular trial

3.10.3.6.2 Grant supported laboratory network B

From July 2003, grant supported laboratory network B was distributed across four countries including Kenya, Botswana, Uganda and Tanzania. The methodologies used by this group consisted of BDS TruCOUNT™ MultiSET™ with CD45 bright gating and BDS FACSCount™ methodology (Table 3.11).

Once again the BDS FACSCount™ users as a group demonstrated good overall performance for all trials, with no outliers reported (Figure 3.27 A-D). Again and in contrast, the performance of BDS TruCOUNT™ MultiSET™ users was poor as reflected by the higher number of outlying results where there were 7 outliers with SDI values >3.0 (Figure 3.27 E-F).

BDS FACSCount™ users A-D



BDS TC/MS CD45 bright gating users E-F

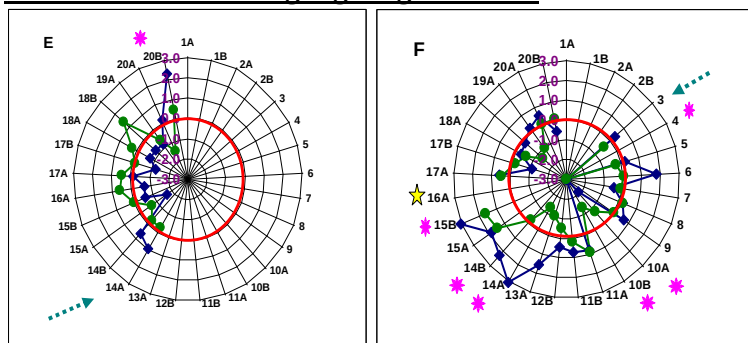


Figure 3.27: Grant supported network “B” Africa Programme. SDI values obtained from representative African laboratories.
The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green ●) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. *Represents outliers, while ★ represents non-submission for a particular trial

3.11 Investigation of laboratories with outlying results

This included error identification with appropriate feedback and corrective action investigation within a SA-NHLS laboratory and an investigation of an African laboratory with multiple outlying results.

3.11.1 Evidence of corrective action

This included error identification (by the CD4 AFREQAS coordinator), with appropriate feedback and corrective action investigation within the SA-NHLS antiretroviral laboratory programme.

3.11.1.1 Investigation of a laboratory with a single outlier

A: To monitor the longitudinal maintenance of quality through participation on CD4 AFREQAS scheme.

B: To investigate a laboratory within the SA-NHLS antiretroviral laboratory programme, with error encountered in the generation of absolute CD4 counts. This required the investigation of imprecision of pipetting through the evaluation of FCR monitoring.

1. Requested the flow count rates (FCR) to be recorded from the laboratory on 32 sequentially analysed samples.
2. Two instruments were investigated with respect to FCR and absolute CD4 count generation.
3. One set of a carousel of 32 samples were run, first on Epics® XL-MCL™ (XL1) and in second instance run on Epics® XL-MCL™ (XL2).

A: To monitor the longitudinal performance of quality through participation on CD4 AFREQAS scheme.

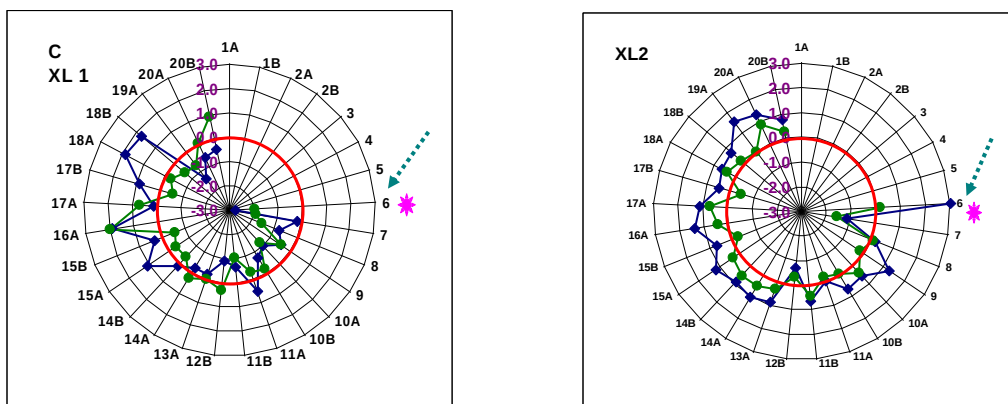


Figure 3.28: A NHLS laboratory with an outlier on Trial 6 material run on Epics® XL-MCL™ (XL1) (C) for AFREQAS participation and on Epics® XL-MCL™ (XL2) second instrument in laboratory. The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green ★) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. ★ Represents outliers, while ★ represents non-submission for a particular trial

B: To investigate a laboratory within the SA-NHLS antiretroviral laboratory programme, with error encountered in the generation of absolute CD4 counts. This required the investigation of imprecision of pipetting through the evaluation of FCR monitoring.

This analysis was done on 32 fresh routine samples at the laboratory (Table 3.13-3.14 and Figure 3.29).

Table 3.13: Descriptive statistical results on FCR data

	FCR (XL1)	FCR (XL2)	% Similarity
Number of values	32	32	32
Minimum	50.8	14.7	61.62
Median	57.5	36.4	82.1
Maximum	106.1	68.6	88.4
Mean	58.8	37.0	81.6
Std. Deviation	9.3	7.1	4.2
Std. Error	1.64	1.26	0.7
%CV	15.8	19.3	4.9

Table 3.14: Outliers removed from XL1 and XL2

	FCR (XL1)	FCR (XL2)
Number of values	32	32
Mean	57.0	36.7
Std. Deviation	3.3	1.64
%CV	5.8	4.4

With the outliers removed, brought the %CV's to the acceptable %CV of 7% or less.

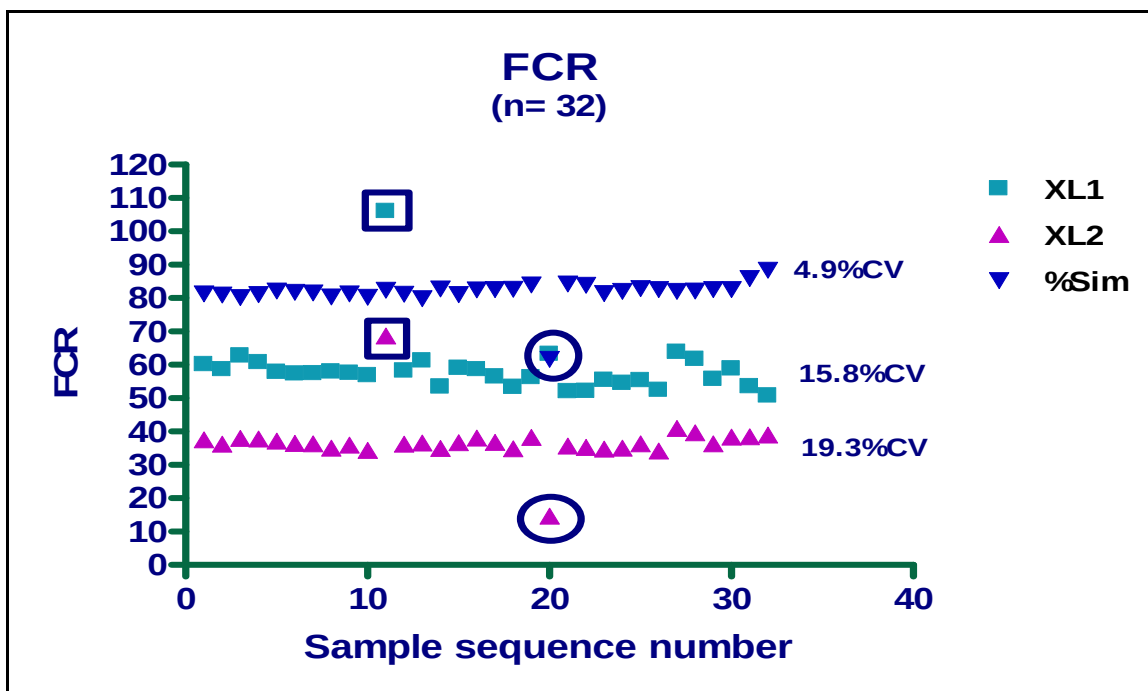


Figure 3.29: FCR sequence plot showing two pipette errors

Table 3.15: Explanation of outliers

Problem encountered	Reason
Outlier on both XL1 and XL2	Error of pipetting
Outlier on XL1 but not on XL2	Error of instrument
Widened %CV on XL1 but not on XL2	Error of fluidics on instrument, needs servicing

3.11.1.2 Corrective action feedback

An outlier on CD4 absolute counts was noted on Trial 6 which fell outside the acceptable 2SD limits from submitted results. This SA-NHLS CD4 ARV laboratory participated on the CD4 AFREQAS scheme. The laboratory was subsequently contacted to investigate the imprecision of pipetting through the validation of the FCR monitoring exercise (Table 3.13 and 3.14).

The error identified from this SA-NHLS CD4 ARV laboratory was due to imprecise pipetting (Table 3.15 and Figure 3.29). With corrective action feedback to this laboratory, additional training and operator pipetting skill validation was performed by the technologist who subsequently obtained a 3%CV which fell within acceptable limits. This technologist has since had a consistently good pipetting record as can be seen from Figure 3.28, with no outliers recorded for all subsequent trials.

3.11.2 Investigation of a laboratory with multiple outlying results

To aid implementation of a more reliable technology in an African site with multiple outlying results participating on the CD4 AFREQAS scheme. A comparative study between their predicate method (BDS FACSCalibur MultiSET™) versus a proposed new technology (BDS FACSCount™) was performed. For this purpose, both local samples (n=43) and a panel of retrospective CD4 AFREQAS EQA samples (n=20) for assessment of precision on CD4 abs counts and CD4%Ly for accuracy assessment (May-June 2006). Fresh and CD4 AFREQAS samples were prepared and analysed at the site using both the reference instrument and the new instrument.

3.11.2.1 Comparison of local samples

Forty three fresh blood samples from the laboratory were prepared and analyses on both the BDS FACSCalibur™ and the BDS FACSCount™ instruments. Samples with CD4 absolute counts ranging from 65.0 to 1617 were included in the study, with the majority of samples with a CD4 absolute count of 784.0 (Table 3.16 and Figure 3.30).

Table 3.16: Descriptive results of fresh samples

	BDS FACSCalibur™	BDS FACSCount™
Number of values	43	43
Minimum	65.0	72.0
Median	784.0	717.0
Maximum	1617	1589
Mean	756.4	806.0

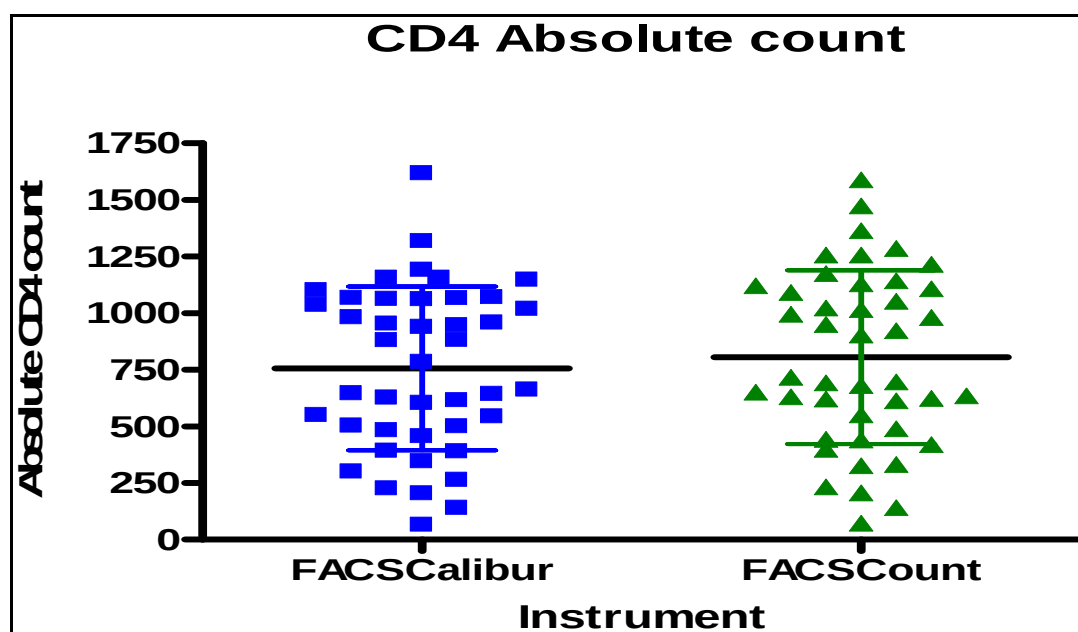


Figure 3.30: Scatter plots describing absolute CD4 counts obtained with BDS FACSCalibur™ and BDS FACSCount™ methodology on fresh blood samples.

(i) Bland-Altman plot

Bland-Altman analyses were performed to assess bias on CD4 absolute count results and determine agreement between two methods (BDS FACSCalibur™ and BDS FACSCount™).

The bias between the two instruments was high (-49.6 cells/μl).

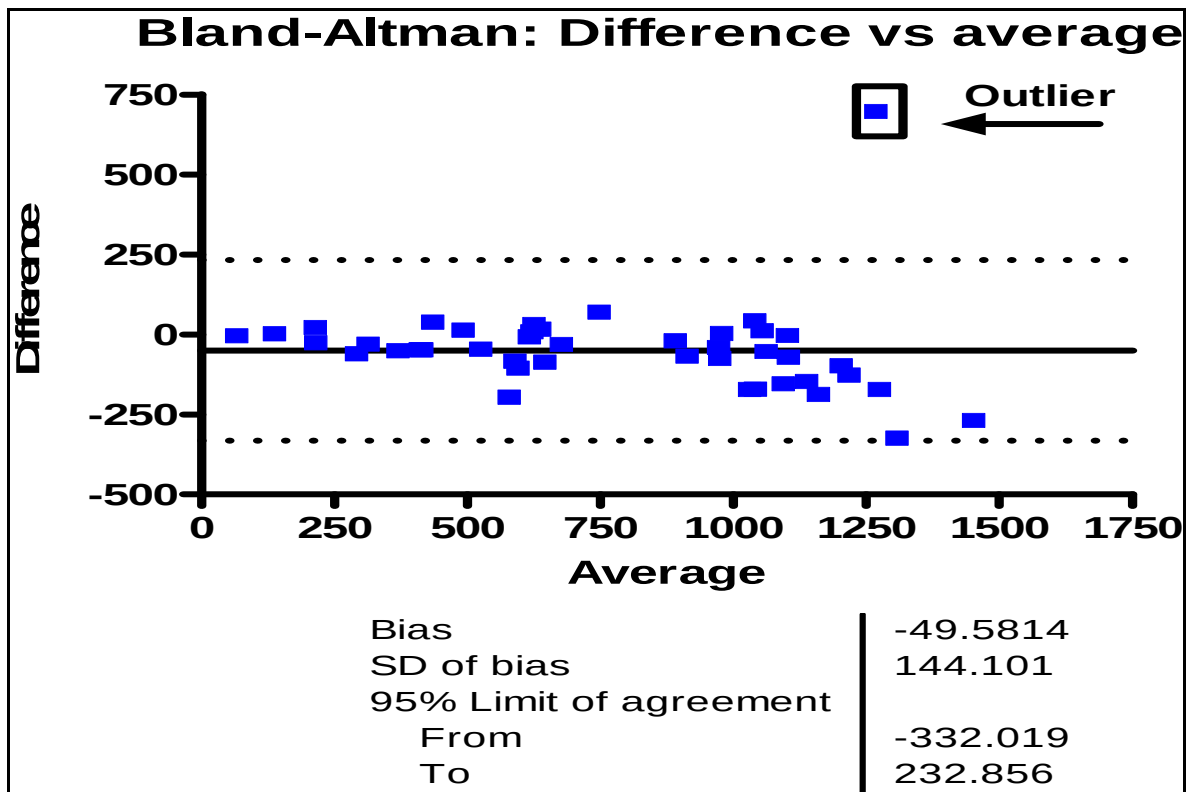


Figure 3.31: Bland-Altman showing bias between BDS FACSCalibur™ and BDS FACSCount™ CD4 enumeration.

One outlier was identified as a sample with a CD4 count of 1617 (BDS FACSCalibur™) and 923 (BDS FACSCount™), i.e. a difference of 694. With this outlier removed, the bias changed to -67.2±86 with 95% limits of agreement -236 to 102.

(ii) % Similarity sequence plot

The percentage similarity plot was also used to assess the bias between the two methods because of a wide range of clinical samples tested (the % similarity smoothes the differences across a wide range of results). The percentage similarity value is represented in a sequence plot to identify outliers over the range of absolute values (Figure 3.32A). Outliers are interpreted clinically to ascertain if the result was clinically significant.

Two outliers identified on the % similarity sequence plot between the BDS FACSCalibur™ and BDS FACSCount™ were not clinically significant (1st had a CD4 count of 482 versus 680; bias of

198; 2nd had a CD4 count of 1617 versus 923 with a bias of 694). Excluding this sample changed the mean %similarity to 104±4.6% with a %CV of 4.5% (precision of difference).

Percentage similarity values were also represented as a Gaussian frequency distribution graph (Figure 3.32B). The distance of the peak from the ideal 100% similarity line (0% difference), indicates the bias between the two methods.

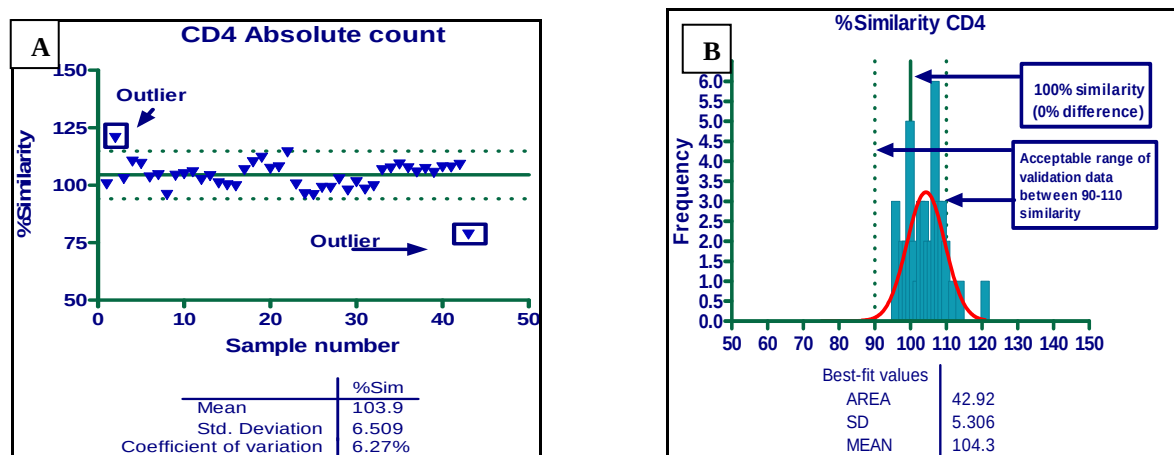


Figure 3.32: (A) % Similarity sequence plot indicating the ideal mean (100%) and 2 SDI limits (90-110%) with 2 outliers identified and (B) a frequency distribution plot.

13.11.2.2 Precision analysis of existing (predicate) and proposed new methodology

Twenty fixed whole blood CD4 AFREQAS identical samples were sent to the site for CD4 testing using both methods (BDS FACSCCount™ and BDS FACSCalibur™) to assess precision of the methodologies.

Table 3.17: Descriptive statistical results on fixed CD4 AFREQAS samples

	BDS FACSCalibur™	BDS FACSCCount™
Number of values	20	20
Minimum	495.0	502.0
Median	703.5	547.5
Maximum	1275	705.0
Mean	782.8	559.5

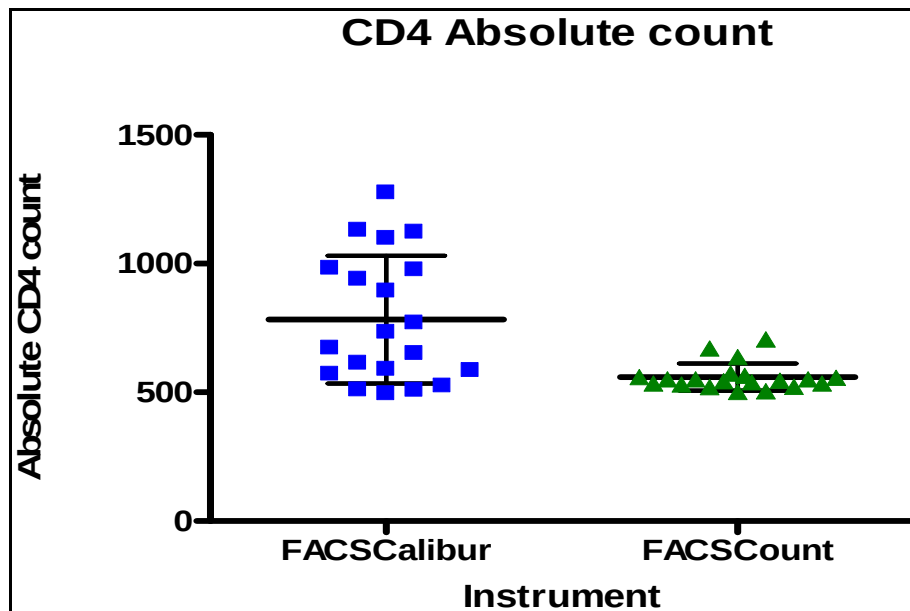


Figure 3.33: Scatter plots describing absolute CD4 counts obtained with BDS FACSCalibur™ and BDS FACSCount™ methodology on fixed CD4 AFREQAS samples.

Results from the fixed CD4 AFREQAS samples showed very poor correlation between methodologies.

Subsequent to helping implement a more reliable methodology in an African site with multiple outlying results, participating on the CD4 AFREQAS scheme, and with ongoing corrective action feedback, this site is using both instruments in their routine laboratory. To date, they are participating on the CD4 AFREQAS scheme. Both instruments are generating good results and this site is a consistent good performer, testimony to the impact of participation.

4.0 DISCUSSION

Funding for the global AIDS crisis has increased exponentially from US \$260 million in 1996 to 10 billion USD in 2007. This is nearly a forty times increase with funding coming from major AIDS financing institutions including the World Bank, United States president emergency plan for AIDS relief (PEPFAR), the global fund to fight AIDS, TB and malaria and the Bill and Melinda Gates Foundation (30).

However, it is important to recognize that financial support from the developed world and charities for health related activities, and more specifically for laboratory capacity and infrastructure, is not the only solution. Africa and other third world countries must be allowed to take ownership and leadership in this regard (105). This requires acknowledging, from both funding agencies and recipients of the funding, that empowerment comes not only with funding (106). Appropriate and quality use of technology and adequate skills-transfer to local, district and national levels empowers local agencies and programmes and secures sustainable, good laboratory practice for adequate support of HIV/AIDS treatment programmes in a resource-poor Africa (71, 105, 107, 108).

The World Health Organisation (WHO) African Region (AFRO) was established in 1996 to provide guidance, technical support and expertise in laboratory technology in the health system (109, 110). National reference laboratories were established through the WHO effort and given a mandate to ascertain the quality of CD4 testing used at their central and district laboratories of their national networks (111, 112). However, despite these efforts to strengthen laboratory capacity in the African region, challenges still remain; laboratory services need strengthening across the region. Christoph Larsen outlines the scenario in his recent review (108), underlined by others as well (30). Generally Larson and others (71, 108, 112-114) describe the factors contributing to these challenges including insufficient funding, lack of national policies and plans for laboratory services, poorly trained staff, weak laboratory infrastructures, old and inadequately serviced equipment, lack of essential reagents and consumables and poor supply of support services like electricity. Where there are laboratory

services, very limited quality assurance including internal and external quality control are described. Further, the challenge facing most African countries lies in developing comprehensive national laboratory procedures for standardization of testing, as well as reagent and instrument procurement. Simplified but standardised procedures are therefore widely needed with more efficient delivery of supplies and instrument manufacturer support. Acquisition through training and most especially, retention of skills also plays a vital role (23, 71, 108, 114). Most countries experience difficulties in setting up laboratories and supporting training facilities to ensure quality testing beyond basic training for technicians. This difficulty results in limited levels of skills available in the countries, with few laboratories being supervised by senior technologists and pathologists due to the brain drain experienced across the health sector (71, 111, 114).

With the roll out of widespread treatment programmes (30, 44), CD4 testing in Africa has increased as well (19, 30). A quality assurance scheme is the backbone of quality laboratory testing. CD4 lymphocyte enumeration is no different in this regard. The need for quality testing, recognition of the impact of EQAS participation and feedback on improved performance of laboratories participating on such programmes, (15, 17, 18, 115, 116) and the absence of regional EQA initiatives, prompted a collaboration between the WHO and the University of the Witwatersrand. This initiative helped to establish a regional EQAS to nurture quality CD4 testing in the region (21, 22). By implementing this Regional African External Quality Assessment Scheme, namely the CD4 AFREQAS (21) quality of CD4 testing in the region could be assessed and potentially improved, using the EQAS as platform to help identify specific skills deficiencies of laboratory CD4 testing (in individual laboratories or across national laboratory networks) and establish structures for further training (21, 22). To build further capacity, the CD4 AFREQAS additionally implemented structures for setting up African national EQAS networks in countries (21). This tiered approach to decentralise distribution of EQA in the region, enables interested African countries to actively participate in the delivery of their local EQAS (taking ownership of their national EQAS) whilst

being supported under the regional AFREQAS umbrella. Five country programmes were established and supported in this way including South Africa, Botswana, Namibia, Lesotho and Zimbabwe. Zambia, Ghana, Nigeria, Swaziland Kenya and Malawi have subsequently expressed interest and requested CD4 AFREQAS support for development of their national EQA programmes as well. Other non-country networks have also joined the CD4 AFREQAS initiative including clinical study (grant based) groups like the International Vaccine Initiative (IAVI) and United States president emergency plan for AIDS relief (PEPFAR).

The performance of established methods and technologies and how these technologies are used in the hands of experienced personnel, largely in the first world, has been established (28, 84, 104, 117-120). Although there are numerous one-off validations comparing a multitude of CD4 technologies in an African setting (52, 121, 122), Only a handful of publications review actual between-laboratory precision and review overall performance of African CD4 laboratories (21-23). The establishment of the AFREQAS offered a unique opportunity to review the quality of CD4 testing not only of individual African laboratories but within groups of laboratories using the same CD4 technology as well as noting the performance across national schemes (relatively under-resourced) or other networks (relatively well resourced grant supported groups). This performance could either be assessed at specific time points (i.e. within a single trial and be comparable to a single multi-centre validation of laboratories) or the pooled data used retrospectively to review longitudinal cumulative performance. Such analysis offered insights into the manner in which African laboratories use (effectively or not) different CD4 technologies and/or methods over time and what kind of problems typically hinder implementation and delivery of quality services.

During 20 trials of CD4 AFREQAS over a period of 4 years, the overall participation rate was excellent with average 91.8% participation and an average non return rate of 8.1%, testament to established recognition by regional participants of the need for quality assessment (19, 30, 71, 107,

111, 112, 114, 123). Overall, reasons for non-submission of results were consistent with the findings of Larsen (Appendix C Table 3.18). These included lack of basic essential equipment, staff shortages and poor technical skills, lack of laboratory consumables and reagents, the lack of supplier and vendor availability for support and maintenance of equipment, use of incorrect pipettes and lack of instrumentation for calibration, unreliable power supplies, and poorly developed communication transport and custom clearance infrastructure. Amazingly, a dead donkey also lead to failure of submission of results in a single Zambian site! (See Appendix D1, supplementary data for details of other reasons for non-submission of results).

The average precision of all participants overall, across 20 trials, was noted to be 11.9CV% for absolute CD4 counts and 10.8CV% for CD4%Ly reporting. This performance is similar to the overall precision (%CV) reported on the UK NEQAS scheme across 23 trials (from January 2001 to February 2005) of 13.1CV% absolute CD4 counts and 13.4CV% for CD4%Ly reporting (here, the UK NEQAS scheme had a response rate of 89.7%) (93). The National Institute of Allergy and Infectious Disease (NIAID) Division of AIDS Quality Assurance (QA) Programme has further reported similar improved precision over a two year period (82). Details of precision within specific trials can be seen in Table 3.6.

Participation on the CD4 AFREQAS scheme has improved individual and between- laboratory variation and identified problems experienced by participating laboratories; the findings are very similar to findings reported in other studies (17, 18, 24, 115). Sometimes dramatic (worsened) changes in the precision were noted when new participants joined the programme, which subsequently returned improved to previous levels of precision of the group when these new participants became more experienced (see Figures 3.6-3.8). Such reduction in %CVs over a 9-year period precision (%CV) was previously reported by UK NEQAS (17), for absolute CD4 values from 15 %CV to <5 %CV (17). Health Canada (QASI) has also shown a decrease in precision

(%CV) over five surveys, decreasing from 7.2% to 4.7% and from 14.2% to 8.8% for CD4%Ly and absolute CD4 counts, respectively (18).

Two levels of CD4 count material were shipped in most CD4 AFREQAS trials (see Table 2.1 for details). With the “normal” and “low” value CD4 EQA material, similar findings were reported in other studies (17, 18), with overall poorer precision with “low” count material than “normal” count material been noted amongst CD4 AFREQAS users. For “normal” count material 10.9% and 8.1% for CD4 abs and CD4%Ly was noted and for “low” count material 14.2% and 17.0% for CD4 abs and CD4%Ly was noted (Figure 3.4).

The precision between laboratories, overall and within groups of laboratories participating on the same trial is shown in Figures 3.6-3.8.

As reported in previous studies (58, 120, 124) the BDS FACSCount™ group showed very good between-laboratory precision with a mean %CV of <7% noted over twenty trials (Figures 3.6, 3.8, 3.9A, 3.10, 3.12). In only one trial (Trial 3) was poor precision seen, with a 13%CV noted. This was due to one instrument failing and requiring a service. Post servicing of this instrument, for all subsequent trials, the precision of the group remained excellent below <10%CV (Figure 3.6). Overall accuracy and precision was also excellent as evidenced by the tight Mean SDI and narrow SD (of the Mean SDI) noted in the retrospective longitudinal analysis (Figures 3.9, 3.10, 3.12). This excellent performance was maintained irrespective of whether “normal” and “low” material was used; %CV of 5.8% and 6.4% were recorded respectively across 20 trials. Of note especially, this excellent performance was noted across all networks, irrespective of origin (noted overall across all FACSCount™ users and that within country- or grant-supported networks).

One error however, common to several FACSCount™ users, was the incorrect submission of CD4% of T cells results instead of the clinically required CD4%Ly values (noted in 16% of FACSCount submitted results). This problem has been attended to by the manufacturers, BDS, and improvements (i.e. new FACSCount™ software for CD4%Ly and absolute CD4 counts) have been

released and validated (125). Another occasional error was related to incorrect pipetting and/ or the use of poorly calibrated pipettes, also reported elsewhere (58). In this instance, the impact of advice given to improve pipetting skills by focusing on within-laboratory precision of individual technicians or advice on calibration of pipettes proved to be invaluable to these laboratories.

There was evidence of several problems related to use of TruCOUNT™/MultiSET™ (TC/MS) algorithm based method (also from BDS) from as early as Trial 1, with poor performance noted intermittently across 20 trials. The poor precision amongst this group was evident both within single trials (Figures 3.6-3.8) as well in the retrospective analysis of pooled SDI data (Figures 3.9B, 3.10, 3.11, 3.13, 3.14, 3.21E-H, 3.24D-F). Contributing to the poorer precision of this group was the dramatic increase of participants (89%) who joined the scheme between Trials 9 and 12. This increase in participants corresponded with a dramatic fall of precision between these users during this period. Here the precision (%CV) between laboratories reporting TC/MS-generated absolute CD4 counts fell from <10% to 26%, suggesting that most new users enrolled between Trials 9 and 12 were not sufficiently trained and largely unfamiliar with the TC/MS technology they were using. During feedback, most new TC/MS participants reported unfamiliarity with the MultiSET software and inability to manipulate the gating and algorithm. Further application training was advised in order for laboratory staff to adequately apply the algorithm and perform appropriate and necessary gating. Attention to pipetting precision was also found to be contributing to the poor between-laboratory precision. In this instance, advice given to improve pipetting skills by focusing on within-laboratory precision of individual technicians (skilled reversed pipetting a necessity) as well as advice on calibration of pipettes proved to be invaluable to these laboratories. Bead rate monitoring (84, 98) was also advised for ongoing within-laboratory quality monitoring. By trial 16 (~7 months later) the impact of additional training and feedback was noted with precision between laboratories using the TC/MS system once again falling in line with expected performance (104) and returning to the pre-Trial 9 TC/MS between-user precision of ~10CV%, remaining as such until

Trial 20. The findings of the TC/MS users highlights the learning effects per trial of participation and that corrective action interventions are proportional to the time of participation on the CD4 AFREQAS scheme (17, 18, 24, 115). Interestingly, in spite of relatively better resourced laboratories and access to training, laboratories participating within the grant-supported networks also did not do well as a group. This finding highlights the fact that investment in training cannot be over emphasized and that algorithm systems, although sold as “automated” systems, still require training and insight for adequate operation (21).

Impact of feedback during EQAS participation was demonstrated in the context of performance of the FACSCount™ and TC/ MS™ users as a group. In one instance, specific advice was offered to a participating laboratory whose current predicate method was TC/MS™ but whose performance had been consistently poor with majority of results falling outside of 2SD of the pool mean (Figure 3.26.E). The participating laboratory was subsequently advised to switch over from their predicate TC/MS™ system to the FACSCount™ system if they wished to improve reproducibility and accuracy of their clinical reporting. Although a bias of -49.6cells/μl noted between methods in the validation switch study performed for the site (Figures 3.30- 3.32), the site was nonetheless advised to switch from TC/MS to FACSCount™ for their routine testing to ensure more reliable CD4 enumeration by the laboratory in the long run. This decision was made based on the marked improvement of precision noted when using the FACSCount™ system (Table 3.17, Figure 3.33). The site was further advised to notify their attending clinicians of the implications of a one-off bias in reporting CD4 and the implications on treatment related decisions. The value of training was strongly emphasized and included improving pipetting skills by focusing on the within-laboratory precision of individual personnel and accurate calibration of pipettes, as well as understanding and use of the MultiSET™ software for correct gating technique. This intervention proved to be invaluable to this site. From Trial 21, the impact of feedback and additional training was noted: this site has used both methodologies in the laboratory to date and has subsequently become a consistent

reliable performing site, with all submitted results being within 2 SD of the trial pool mean, for both their TC/MS™ and FACSCount™ systems (data not shown).

Analysis of performance of groups using specific gating strategies supported published data showing that laboratories who use CD45 bright (lymphocyte) gating perform for CD4 enumeration do better than laboratories who do not (82, 115, 126). Data from the AFREQAS TC/MS™ group who used a CD8/CD4/CD45/CD3 protocol with CD45 bright gating (126) performed better (14%CV and 20.8% CV for absolute CD4 counts and CD4%Ly respectively) than those laboratories that used CD3/CD4/CD8 with primary CD3 gating (127) for analysis (precision of 21.4%CV and 41.7CV% was noted for absolute CD4 counts and CD4%Ly respectively). This highlights the importance of adherence to international guidelines (2, 128) when such systems are used. As noted within their BDS counterparts (i.e. the FACSCount™ user group), these participants also incorrectly reported CD4% of CD3+ T cell values instead of the required CD4%Ly. These results were identified as outliers and in turn affected the precision of the group (Figures 3.6, 3.9A, 3.10, 3.12). Although frequently after feedback, some laboratories understood the difference of reporting and no longer reported CD4% T cells in error, other continued to incorrectly submit the CD4% T cells values.

The laboratories using the SP PLG-CD4 method with manual gating showed overall very good between-laboratory precision, with an average %CV of <9% for absolute counts and <5% for CD4%Ly noted over the period of participation (Figures 3.6-3.8). This precision has been shown in other studies (28, 72, 118, 129). The proactive use of bead count rate monitoring with the PLG-CD4 method helped to ensure reliable between-laboratory precision (84, 98). In addition, a major contributing factor was that the PLG-CD4 users were given ongoing supportive training by the SA-NHLS CD4 reference laboratory and Beckman Coulter South Africa. This training included on-site and off-site workshop training, consolidated into website based and opportunities for hands on

training in an established accredited facility reference laboratory (130). The excellent performance of this group highlights and emphasises the importance and value of use of proactive quality control and ongoing consolidated training support for laboratory personnel (23, 84) in ensuring sustainable quality of CD4 reporting. Similar excellent within and between laboratory precision of laboratories using SP PLG CD4 has been reported as far afield as the Caribbean (72).

Some minor problems that occurred included incorrect gating protocol (not the standardised NHLS protocol), incorrect reporting of CD4% of the white cell count result instead of reporting a CD4%Ly and occasional transcription errors. With corrective action intervention, all problems were easily rectified. Most especially of note with the SP PLG-CD4 method was the consistent reliable precision of CD4%Ly values reported by these users. Although CD3 is not included in the protocol to identify T cells and exclude monocytes as is used in other T cell enumeration methods (46, 128, 131, 132), the excellent precision of the users in this group confirms existing reports that show that CD4 and side scatter measurement (without CD3) is sufficient to identify CD4 lymphocytes and adequately exclude contamination with monocytes (53, 118, 133-135).

The impact of CD4 AFREQAS feedback to a SA-NHLS laboratory with a single outlying result caused largely by pipette error is noted in Figure 3.20C. Here, corrective action feedback, consolidated with the integrated system of initial site validation (99), proactive quality control (84, 96) on-site and offsite workshop training in an established accredited facility reference laboratory (136), website based training (130) has made a huge impact on excellent CD4 service delivery of the NHLS laboratories in South Africa (84).

In contrast to the guideline based TC/MS also using CD45/3/4/8 with algorithm based analysis, a small group of BC Flow-Count™TetraONE™ users showed consistently good performance, similar to that noted elsewhere in a multi-site study (119). Despite manual gating used by these sites, across the twenty trials, no problems encountered with this method and there were no outlying results noted (Appendix D). However, all the sites were within South Africa and had direct access to

training within the National Health Laboratory Service from the Johannesburg NHLS Reference laboratory, as well as access to training from Beckman Coulter South Africa. Once again, this emphasizes the importance of the role of training and insight into testing, as well as manufacturer support, in the delivery of quality CD4 testing.

Although the number of users was small, as a group, the laboratories using the Volumetric counting/ methodology from Partec® method did not perform well. This group showed generally poor between-laboratory precision across 20 trials with an average precision (%CV) of 27% noted for absolute CD4 count reporting (Figures 3.6, 3.8). Evidence of problems within the Partec® user group was initially evident on review of this group's within trial precision (%CV) on individual trials (Figures 3.6, 3.8). The poor precision of these Partec® users as a group was further confirmed through the retrospective cumulative analysis of the pooled SDI results of the group over 20 trials (Figures 3.9D, 3.23C-H, 3.24 G-H). Compounding the poor performance, the number of Partec® participants increased by 43% during Trials 18 and 19, corresponding with a dramatic fall of precision between these users during this period. Here the precision (%CV) between laboratories reporting absolute CD4 counts fell from <13% to 34% suggesting that most new users enrolled between Trials 18 and 19 were not sufficiently trained and largely unfamiliar with the technology they were using. During feedback, most new Partec® participants reported that they had received little training and technical support from the Partec® manufacturers. Although it was acknowledged that access to training was difficult for these users, further training was strongly advised to improve quality in these laboratories. Attention to pipetting precision was found to be one of the contributing factors leading to the poor between-laboratory precision. In this instance, advice was given to improve pipetting skills (once reverse pipetting for accurate volumetric testing) by focusing on within-laboratory precision of individual technicians (practicing pipetting water and blood) as well as calibration of pipettes, especially necessary in the context of volumetric testing. However this proved to be a problem as some laboratories did not have basic laboratory equipment. For example

a basic analytical balance was lacking at most sites for validating pipettes. Further bead rate monitoring (98) was also suggested as a further additional internal quality control of pipette error (although technically the Partec system uses volumetric methodology and does not require beads, BCR monitoring was suggested purely to monitor pipetting error). All advice, unfortunately, had no impact on performance. By trial 20, (~2 months later), precision (%CV) of this group continued to be much worse than the overall global precision of the pool of users, with an average precision of 28%CV noted. Some sites recognised the need to swap technologies and after a period of poor performance and consultation with CD4 AFREQAS, one site changed their method to the BDS FACSCount™ (with consequent excellent precision performance on the CD4 AFREQAS scheme noted with other FACSCount™ users). This again highlights that systems with minimal training requirements can facilitate delivery of good results in settings where training resources are limited. Typically, these instruments, especially the low end models, are not able to generate CD4%Ly values. In this group, 3.3% of users incorrectly reported CD4% of T cells results instead of the clinically relevant values CD4%Ly results. As noted with participants of the CD4 AFREQAS using either FACSCount™ and TC/MS™ using primary CD3 gating, laboratory personnel using Partec® equipment do not know the difference between required CD4%Ly values and the CD4% of T cells reported on a Partec®. Appropriate corrective feedback helped to ensure that this error was not repeated. The problems related to use in a resource poor setting has been noted by others. In a study done by Lynen L. et al it was demonstrated that it was imperative that intensive training of the staff was conducted to obtain reliable results (137). Another study done by Pattanapanyasat K et al showed that technical training was an absolute requirement for accurate CD4 counting (56). In Malawi, local laboratory staff reported that the Partec® instrument procedures simple to run and the instrument easy to operate after training . Others have reported that bead calibration of volumetric testing is crucial to ensuring precision and accuracy of these instruments (54, 121).

Overall, the participants using this volumetric method were unable to match the performance of the technology reported elsewhere (117) However and in striking contrast, the performance of a

preference laboratory participating from Germany was excellent. This laboratory had requested joining the CD4 AFREQAS programme as a reference site for two Mission Hospitals it supported in Africa. Excellent within-laboratory performance was noted from this German laboratory (Appendix D supplementary data), once again confirming that insight into instrument operation and knowledge of maintenance procedures is of vital importance in ensuring optimal performance of the technology.

The number of dual platform users was small (7%) amongst the CD4 AFREQAS users, with varying levels of precision was noted. Those laboratories that received good technical support, and training from respective suppliers or within their network, showed good precision performance across the trials. As with the group using the equivalent single platform system, the group using the Beckman Coulter DP TetraONE™ (CD45/4/8/3) users who received good technical support and training, were sites within South Africa that had direct training within the National Health Laboratory Service and access to training from Beckman Coulter South Africa. These sites outperformed those African laboratories who did not receive the support, once again emphasising the importance training and manufacture support in the delivery of quality CD4 testing.

However, and once again, laboratories who did not receive the support and training performed less well. Laboratories that used traditional DP testing but with abbreviated panels or algorithm based automated software (MultiSET™) for analysis performed less well (Appendix D supplementary data). Problems that contributed to this poorer performance included use of total lymphocyte count in the traditional DP system, a known variable parameter in the calculation of absolute CD4 counts between laboratories (2, 127, 128). Others reported difficulties with gating and manual overriding of algorithm software with use of MultiSET™ software.

The best performance amongst the group of laboratories using dual platform methodology was shown with amongst the DP Panleucogated (PLG-CD4) users where no outliers were noted across 20 trials. The improved precision PLG-CD4 method despite a DP format, is based on established

good precision of white cell counts between laboratories (2, 83, 134, 135) and avoids use of the conventional guideline based Total Lymphocyte count used in traditional DP testing (132). Here, as reported elsewhere (117), individual laboratory performance and precision between laboratories, was shown to be equivalent to that noted with single platform testing.

A retrospective statistical analysis was performed comprising all individual standardised SDI results, pooled across 20 trials, to assess mean SDI of laboratories grouped according to the CD4 methodology used (21) (Figures 3.10- 3.15). The three most commonly used methodologies over the twenty trial are analysed using this model (Figures 3.10- 3.15), including the BDS FACSCount™, the BDS TC/MS™ and SP PLG-CD4 user groups. In contrast to the precision (%CV) within individual trials where data was trimmed (Figures 3.6-3.7), this retrospective analysis includes all results, as well as outliers, to give an overview on the true precision between laboratories using the CD4 methodology. It further facilitates insights into how CD4 technologies are used, which technologies are best used, how much error is associated with a use of particular technology and further, based on the latter, what the training needs are. As mentioned previously, the mean SDI demonstrates longitudinal accuracy and the SD (of the Mean SDI) gives an idea of long term and ongoing precision. The group of laboratories that showed a mean (SDI) close to 0 and SD (Mean SDI) within the range of 0 - 1 were demonstrated to have the best accuracy and precision. In this analysis, two groups emerged: one group showing laboratories with tighter between laboratory precision suggesting less additional training needs (predominated by FACSCount™, SP PLG-CD4 and DP PLG-CD4 users) and a second group showing laboratories with wider between laboratory variations with more training needs (predominated by users of TC/MS technology) . The details of this analysis can be seen in (Figures 3.10- 3.15).

As confirmed in the individual trial analysis where within trial precision of the group was excellent (Figure 3.9A and C), the laboratories using the BDS FACSCount™ technology as well as the SP PLG-CD4 user group showed mean SDI values close to 0 and SD SDI values within the range of 0 -

1, indicating that these laboratories were able to consistently submit reliable and accurate results. Confirming the poorer performance of TC/ MS users noted trial by trial (Figure 3.9B) the laboratories within the BDS TC/MS group performed less well and showed a wider variation of results. Although this group showed acceptable average accuracy, the spread of the data within the SDI range, as reflected by the SD (Mean SDI), confirmed the overall poorer precision of this group (with $\pm$ Mean SDI range falling between 1 - 2).

In response to internal and external pressure to combat HIV/AIDS, the South African government released its Operational Plan for the Comprehensive HIV and AIDS Care, Management and Treatment for South Africa in November 2003 (138, 139). In support of this programme, there are currently more than 70 SA-NHLS laboratories offering CD4 enumeration SA-NHLS has standardised CD4 testing with the simpler SP protocol (PLG-CD4). The excellent performance of this network in comparison with other groups on the CD4 AFREQAS, has been ensured with use of novel proactive internal quality control, viz. BCR monitoring introduced to exclude pipetting errors (84, 98), together with active training programmes including workshops organised by the NHLS Johannesburg Reference laboratory, as well as website training access (130). Despite the need for some operator input and use by personnel with little or no flow cytometry background, notably the performance of this group compares well with the excellent performance of the FACSCount users as a whole (where minimal input is required from the operator).

Namibia also has standardised CD4 testing across five national health laboratories to SP PLG-CD4 following the implementation of their national ARV treatment programme. Across the trials, the performance of the standardised SP PLG-CD4 participants was good but not as impressive as the South African NHLS laboratory network. As noted in the SA-NHLS programme, occasional outlying values were due to incorrect reporting of CD4% of WBC and/ or the occasional inaccurate gating. Here, performance of the Namibian PLG network (Figure 3.22 A-F) suggests that these laboratories require additional training with a focus on pipetting precision and use of bead count

rate monitoring to improve internal quality control (98) if they are to match the performance of their South African counterparts. Occasional non submission of results was due to personnel shortages; problems of increased staff turnover were frequently reported.

Problems of a similar nature were noted across the Botswana, the Lesotho and the Zimbabwe national networks. Most notably, the lack of standardisation across all three programmes has impacted negatively on the overall performance of these national networks. BDS TC/MS CD45 bright gating system was used in laboratories in all three countries and the performance was generally inconsistent and poor. Similar to sites across the region, and in South Africa, factors contributing to the poorer precision included poor understanding and application of the automated algorithm software especially related to gating and manual identification of relevant populations seen (Figures 3.21 A-H, 3.23 A-B, 3.24 D, 3.26 E-F, 3.27 E-F). The Partec® system was additionally used in Lesotho and Zimbabwe and both national network laboratories showed poor precision with this methodology (Figures 3.23 G-H, 3.24 G-H). The main contributing factor was the lack of training on use as well as limited maintenance and repair of instruments. In spite of corrective action feedback, the Lesotho and Zimbabwean national programmes continued to show wide variability of CD4 results many trials later. Of note however, and in stark contrast within these three networks, is the excellent performance of FACSCount™ users (Figures 3.21A-D, 3.24 A-C, 3.26 A-D, and 3.27 A-D). Here, once again, even though the same logistical difficulties and other political and economic circumstances were present at these sites, the laboratories within the networks using FACSCount™ markedly outperformed their counterpart laboratories, showing excellent between –laboratory results. Zimbabwe was especially affected by local politics and dire economic circumstance where four otherwise excellent performing FACSCount™ sites were unable to submit results for several trials. Other major problems reported from sites across either Zimbabwe, Botswana or Lesotho involved numerous breakdowns following the installation of equipment. Poor instrument manufacturer maintenance and service delivery and lack of funds or

human resource further contributed. In Botswana and Zimbabwean participants also reported major problems with reagents with short shelf life and/or receipt of expired reagents often due to slow customs clearance. In contrast to the South African network, the most obvious differences noted in Namibia, Lesotho, Botswana and Zimbabwe were the lack of active training initiatives and standardization. Use of systems like the BDS FACSCount™ or alternatively, SP PLG methodology with supportive training coordinated through the South African NHLS, could significantly reduce between laboratory precision in Lesotho, Botswana and Zimbabwe, as noted previously on the South African NHLS network (84), the Brazilian programme (124) or in the Caribbean (72).

Although considerably better resourced than their African counterpart laboratories, similar performance of laboratories was observed within the grant supported laboratory networks as that seen in the African national networks. From August 2003, grant supported laboratory network “A” sponsors has been in collaboration with African partners to collect epidemiological data and to build capacity for large-scale clinical trials on HIV/AIDS (140). These trials are taking place in four countries, including Kenya, Rwanda, Uganda and Zambia and further include several trial sites. From July 2003, grant supported laboratory network, designated “B”, acted with US agencies to build capacity for large-scale clinical trials on HIV/AIDS focusing on the interventions to prevent HIV transmission. These trials are taking place across nine sites in four countries including Kenya, Botswana, Uganda and Tanzania.

The performance of these grant-supported networks was reviewed to demonstrate that similar issues and problems emerge within African networks, irrespective of resources and funding. Once again, problems emerge that are largely due to lack of training and instrument maintenance as well as lack of technical support and trained staff leaving because of recruitment of other companies or international posts being available. Neither of networks had standardised methodologies in place. During feedback, participants using the TC/MS system also reported unfamiliarity with the MultiSET™ software and inability to manipulate the gating and algorithm. Attention to pipetting

precision was also found to be contributing to the poor between-laboratory precision. FACSCount users, as in other African networks, as a sub-group, demonstrated excellent performance for all trials. None of the US based grant networks used PLG CD4.

In summary, outliers identified on the CD4 AFREQAS scheme were investigated by the AFREQAS coordinator to help laboratories resolve problems with methodology and/or technical issues. The advantages and strengths of all methods have been demonstrated in this study. The improvement in CD4 reporting found in this study and others (22, 23, 84), confirms that African laboratory technicians are able to use simpler flow cytometry systems that require minimal training or operator input when internal quality controls are in place (28, 58, 84, 120). These include, most notably the BDS FACSCount™ that can be used with automatic pipetting in a low to medium volume throughput laboratory with minimal training or the cost effective SP PLG-CD4 method with a capacity for high volume throughput of CD4 tests per day but requiring some training in flow cytometry and pipetting skills. BDS TruCOUNT™MultiSET™ and Partec® methods have demonstrated poorer between and within-laboratory performance on the CD4 AFREQAS scheme. However, the benefits of training and manufacturer support, as well as the use of proactive quality control systems (58, 84, 96, 98, 99, 120, 124, 130, 141) were clearly demonstrated to have an impact on improving the performance of laboratories using these systems.

5.0 CONCLUSION

The aim of the study was to implement a regional CD4 EQA scheme (CD4 AFREQAS), not only to assess performance of participating laboratories but to provide a platform for improving reproducibility of CD4 reporting both within- and between-laboratories in South Africa and Africa. Further CD4 AFREQAS has provided ongoing teaching through participant feedback evaluation and dedicated training programmes, as well as advocated the use of standardised laboratory protocols necessary to contain costs and maintain high standards of CD4 testing.

With universal access to ARV therapy in Africa, the demand for CD4 testing is on the increase. This study has demonstrated that African laboratories do understand that participation in CD4 AFREQAS can highlight deficiencies in their laboratories. Improvement of performance following corrective action advice and improved quality of their CD4 service delivery in their respective countries is evidence supporting the impact of corrective action feedback. Further, the performance of specific networks within the region (31) has shown that African countries can benefit from implementation of standardised national strategies and plans to support CD4 testing and monitoring. In South Africa especially, through the integrated system of the CD4 AFREQAS scheme, and the use of corrective action feedback, together with on-site and offsite workshop training in an established accredited facility reference laboratory all consolidated into website based training, the impact of standardization is noted and precedent set for establishing excellent CD4 service delivery in an African setting. Guidelines on standardisation of CD4 (and other laboratory testing) in Africa could play an important role in scaling up and establishing excellent service delivery in the region.

This study has confirmed previous reports and firmly establishes which factors contribute to poor performance and has further highlighted which technologies are best used in an African setting. However, investment in additional technical training and support from the flow cytometry

manufacturers cannot be over emphasized. Education of laboratory personnel will ultimately secure and help to sustain service delivery and improve the quality of CD4 testing in the region.

The success of the CD4 AFREQAS scheme is highlighted by the fact that from 195 participants at the end of Trial 20, it had grown to more than 500 registered participants by early 2009. In addition the scheme obtained SANAS (South African National Accreditation System) accreditation in August 2008, so that it conforms to international standards. Therefore the CD4 AFREQAS scheme offers African participants a scheme which is accredited, and internationally recognised as being on a par with schemes such as the UK NEQAS. The difference with the CD4 AFREQAS scheme is that it offers assistance with additional training and corrective action support. To conclude, the scheme has been widely accepted by African participants who have found that the CD4 AFREQAS scheme can help them to implement a better service.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

R14/49 Aggett

CLEARANCE CERTIFICATE

PROTOCOL NUMBER 00-01-07

PROJECT

Quality Assessment programme for Southern Africa

The impact of the development of CD4 External

INVESTIGATORS

H Aggett

DEPARTMENT

Molecular Medicine.

DATE CONSIDERED

04.02.14

DECISION OF THE COMMITTEE*

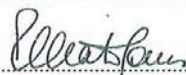
Approved under R & D clearance

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE

04.02.14

CHAIRPERSON



(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr D K Glencross



DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. I agree to a completion of a yearly progress report.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Documentation required for the CD4 AFREQAS scheme**APPENDIX B:**

Registration form

APPENDIX B1**CD4+ Lymphocyte Proficiency Programme
2009**

A new registration form is to be completed for participation in 2009
IMPORTANT: Please put your participant code on the form if you
Already have one.

REGISTRATION FORM-PARTICIPANT CODE:

Pathologist:	
Phone No:	
Fax No:	
e-mail address:	

Flow Analyst:	Contact Person in the laboratory:
Phone No:	
Fax No:	
e-mail address:	

Mailing Details:

<u>Physical Address:</u>	IMPORTANT FOR COURIER DELIVERY
Laboratory Name:	
Institution:	
Room Number:	
Floor Number:	
Department:	
Street:	
City:	
Postal Code:	
<u>Postal Address:</u>	
Laboratory Name:	
Institution:	
Department:	
PO Box:	
City:	
Postal Code:	

The 2009 registration form is for continued participation and to update records.

FAX OR E-MAIL BACK TO HAZEL AGGETT AT:

FAX: +27 11 386 6296

E-MAIL: hazel.aggett@nhls.ac.za

Participant unique code number:

APPENDIX B2



CD4+ Lymphocyte Proficiency Programme

Dear PARTICIPANT,

Thank you for participating in our CD4 AFREQAS programme. Your unique participant code number is the following:

LABORATORY NAME, participant code number is XXX

Participant name and participant code number must be written on the results form

Hazel Aggett
CD4 AFREQAS Co-ordinator
Quality Assurance Department
NHLS

phone: +27-11 - 555 0413
fax: +27-11 - 386 6296
e-mail: hazel.aggett@nhls.ac.za



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Certificate of participation:

APPENDIX B3



CD4+ Lymphocyte Proficiency Programme

We certify that the organisation named below is registered to participate in the CD4 AFREQAS Proficiency Programme for the period:

January 2009 to 31 December 2009

Participant name:

Participant number:

Associate Professor Deborah Glencross
Department of Molecular Medicine and Haematology
Faculty of Health Sciences
University of the Witwatersrand and NHLS
Johannesburg
Email: debbie.glencross@nhls.ac.za
Tel: +27 11 489 8540

Mrs Hazel Aggett
Department of Molecular Medicine and Haematology
Faculty of Health Sciences
University of the Witwatersrand and NHLS
Johannesburg
Email: hazel.aggett@nhls.ac.za
Tel: +27 11 555 0413



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CD4+ Lymphocyte Proficiency Programme

Laboratory Safety

- A. Use universal precautions with all specimens.
- B. Adhere to the following safety practices.
 1. Wear laboratory coats and gloves when processing and analysing specimens, including reading specimens on the flow cytometer.
 2. Never pipette by mouth. Use safety pipetting devices.
 3. Never recap needles. Dispose of needles and syringes in puncture-proof containers designed for this purpose.
 4. Handle and manipulate specimens (e.g., aliquot, add reagents, vortex, and aspirate) in a class I or II biological safety cabinet.
 5. Centrifuge specimens in safety carriers.
 6. After working with specimens, remove gloves and wash hands with soap and water.
 7. For stream-in-air flow cytometers, follow the manufacturer's recommended procedures to eliminate the operator's exposure to any aerosols or droplets of sample material.
 8. Disinfect flow cytometer wastes. Before adding waste materials to the waste container, add a sufficient volume of undiluted household bleach (5% sodium hypochlorite) so that the final concentration of bleach will be 10% (0.5% sodium hypochlorite) when the container is full (e.g., add 100 mL of undiluted bleach to an empty 1,000-mL container).
 9. Disinfect the flow cytometer as recommended by the manufacturer. One method is to flush the flow cytometer fluidic chambers with a 10% bleach solution for 5-10 minutes at the end of the day and then flush with water or saline for at least 10 minutes to remove excess bleach, which is corrosive.
 10. Disinfect spills with household bleach or an appropriate dilution of mycobactericidal disinfectant. **Note:** Organic matter will reduce the ability of bleach to disinfect infectious agents. NCCLS recommendations regarding how to disinfect specific areas should be followed. For use on smooth, hard surfaces, a 1% solution of bleach is usually adequate for disinfection; for porous surfaces, a 10% solution is needed.
 11. Ensure that all samples have been properly fixed after staining and lysing, but before analysis. **Note:** Some commercial reagents employ a single-step, lyse and fix method that reduces the infectious activity of cell-associated HIV by 3-5 logs, however, these reagents have not been evaluated for their effectiveness against other agents (e.g., hepatitis virus). Cell-free HIV can be inactivated with 1% paraformaldehyde within 30 minutes.

Reference

Guidelines for Performing Single-Platform Absolute CD4+ T-Cell Determinations with CD45 Gating for Persons Infected with Human Immunodeficiency Virus

Recommendations and Reports. Vol. 52 / RR-2. Francis F. Mandy, Ph.D MMWR - January 31, 2003-07-06



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CD4+ Lymphocyte Proficiency Programme

March 2008: INSTRUCTIONS TO LABORATORIES

Return Date: Tuesday 25 March 2008

Participant name and participant code number must be written on results form

This material must be stored at 2°C-8°C until analysed.

Potential biohazardous material

Each donor unit used in the preparation of this material was tested by an FDA approved method for the presence of the antibodies to Human Immunodeficiency Virus (HIV-1, HIV-2) and Hepatitis C Virus (HCV) as well as the Hepatitis B surface antigen (HbsAg) and found to be negative (were not repeatedly reactive). Because no test method can offer complete assurance that HIV, HCV, Hepatitis B Virus (HBV), or other infectious agents are absent, this reagent should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen.

The laboratories should use good laboratory practice when handling this reagent.

Process the stabilised human blood product as if you were handling routine blood samples (observing associated safety precautions).

Complete the attached forms and return by fax and if possible e-mail to:

Hazel Aggett
CD4 AFREQAS Co-ordinator
Quality Assurance Department
NHLS

phone: +27-11 - 555 0413

fax: +27-11 - 386 6296

e-mail: hazel.aggett@nhls.ac.za

Please contact Hazel Aggett if you would like the return forms in electronic format to facilitate email submission of results.



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Export permit:

APPENDIX B6



health

Department:
Health
REPUBLIC OF SOUTH AFRICA

Private Bag X828, PRETORIA, 0001, 10TH Floor, House of Trade and Industry (HTI) Building, Cnr Prinsloo and Pretorius Street, PRETORIA, 0001 • Fedlife Building, Cnr Prinsloo and Pretorius Street, PRETORIA, 0001
• Hallmark Building, 226 Vermeulen Street, PRETORIA
Tel (012) 312 0816 Fax (012) 323 0093

(012) 312-3108

Mr JR Mokonoto

(012) 312-0395

J1/2/4/2 No. 75

EXPORT PERMIT

In terms of Section 25 of the Human Tissue Act, 1983 (No 65 of 1983) –

Professor Deborah Glencross
Principal Pathologist, Head Flow cytometry
National Health Laboratory Services
P O Box 1038
JOHANNESBURG
2000
Tel. No.: (011) 489-8541
Fax No.: (011) 484-5812

is hereby authorised to export from the Republic of South Africa –

+/- 250 g

Diagnostic specimens for Proficiency testing, comprising Fixed, stabilized
(non-infectious) whole blood.

to –

SWAZILAND

for – Proficiency testing for laboratory quality assessment programme.

This export permit is subject to the following conditions:

1. The substance shall be imported into the country specified above, within the legal requirements of that country.
2. The substance shall be exported from South Africa and handled in accordance with the provisions of the Human Tissue Act, 1983 (No 65 of 1983), and the regulations made in terms of the Act.
3. The export permit shall not be used for any trade or advertising purposes.
4. This export permit shall expire on 31 December 2008.

 DIRECTOR-GENERAL: HEALTH

Date 2007-12-04

C C Kotzenberg

Professor D Glencross 75

Commercial invoice:

APPENDIX B7

	CD4+ Lymphocyte Proficiency Programme
	South African National Health Laboratory Services Vat Registration Number: 4900103575

COMMERCIAL INVOICE

Senders Address (Shipper):

Recipients Address (Consignee):

Hazel Aggett
Department of Molecular Medicine
Faculty of Health Sciences
Room 3N29
7 York Road
Parktown
Johannesburg, South Africa
2193
Tel: +27-11-4898541
Fax: +27-11-4845812

NO. OF PKGS.	DESCRIPTION OF GOODS	SPECIMEN QTY	PKG. WEIGHT	VALUE US\$
<u> 1 </u>	Biological substance category B UN 3373 (blood or serum or urine or other body fluids or biopsies) Packed in Accordance with IATA Packaging Instruction 650. Fixed stabilised blood containing no animal material and not of tissue culture origin. Transported for diagnostic testing as part of a clinical trial.	<u> </u> mL or <u> 500 </u> g	<u> </u> kg	US \$ 10.00
Total Pkgs: <u> 1 </u> —			Total Pkg: Weight: <u> 0.5 </u> kg	Total Invoice Value: US \$ 10.00

OF NO COMMERCIAL VALUE; VALUE STATED FOR CUSTOMS PURPOSE ONLY.

THIS INFORMATION IS TRUE AND CORRECT.		INTERNATIONAL AWB NO:
SIGNATURE, TITLE _____	DATE _____	_____

Import permit:

APPENDIX B8



DEPARTMENT OF HEALTH
Republic of South Africa

DEPARTMENT OF HEALTH
Private Bag X828 PRETORIA 0001

Inquiries: Mr JR Mokonoto Tel.: (012) 312-0395 Fax: (012) 312 3113

**APPLICATION FOR AN IMPORT PERMIT FOR
BIOLOGICAL SUBSTANCES**

Person applying for an export permit:	
NAME	Professor Deborah Glencross
RANK/POSITION	Principal Pathologist, Head Flow cytometry

Organisation:			
NAME	National Health Laboratory Service		
ADDRESS	P.O.Box 1038	Wits Medical School	
	Johannesburg 2000	Parktown Johannesburg	
TEL. NO.	011- 489 8541	FAX. NO.	011- 386 6296

Specific substance(s) for which an import permit is required:	
SUBSTANCE	QUANTITY
Diagnostic specimens for Proficiency testing, comprising Fixed, stabilised (non-infectious) whole blood.	400 x 3.0ml
Period during which export will take place	January 2007 – December 2007

Contact person and organisation to which the substances are exported:			
NAME: PERSON	Mr Guy Houle		
NAME: ORGANISATION	National HIV Immunology Laboratory		
ADDRESS	LCDC Building Tunney's Pasture OTTAWA, ONTARIO CANADA		
TEL. NO.	+613 946 3238	FAX.NO.	+613 946 3237

Purpose(s) for which substance(s) is (are) to be used. Although detail is not required, the specific purpose(s) must be clearly stated:
Proficiency testing for laboratory Quality Assessment Programme

Result sheet:



CD4+ Lymphocyte Proficiency Programme

Sample Condition: SATISFACTORY/ Unsatisfactory

Reason: _____

Return Date: Tuesday 25 March 2008

Participant name and participant code number must be written in

Participant Name:		Code number:	
Received: (DD/MM/YY)		Date of Analysis: (DD/MM/YY)	

1. The emphasis of these trials is CD4 EQAS, and participants who report only a CD4 absolute count, and CD4 percentage of lymphocytes (e.g. with PLG CD4), are not compromised on this analysis by non submission of CD3 or CD8 results

2. **Please note:** The nature of QA material is such that it cannot reliably generate an ALC on a haematology analyser. It is therefore imperative that your laboratory generates a flow differential (either 45 or LS based), and use the percentage lymphocytes obtained from this differential multiplied by the WCC from a haematology analyser to generate your ALC (absolute lymphocyte count). For DP – lymphocyte users only.

Please tick all the relevant blocks that are applicable to your laboratory / analysis method.

Antibody panels- EXAMPLE ONLY			
Panel	Identify the antibody (CD) and fluorochrome for each tube of the panel		
Tube 1	MAB - FL1	MAB - FL2	MAB - FL3
Tube 2	CD8 FITC	CD4 RDI	CD3 PC5

Participants: Please fill in your monoclonal antibodies below

Antibody panels			
Panel	Identify the antibody (CD) and fluorochrome for each tube of the panel (only use the relevant column e.g. if using a CD3/4/8, then only fill in MAB-FL1, MAB - FL2 and MAB-FL3 in row for tube 1		
Tube 1	MAB - FL1	MAB - FL2	MAB - FL3
Tube 2			

Basic methodology:	
Single Platform	
Dual Platform	

Single Platform:	
Dedicated	Yes No BDS FACSCount
	Other:

Bead product:	
BDS Trucount	
BC FlowCount	
Other:	

Pipette Method:	
Manual	
Use same pipette for blood and beads with normal pipetting	
Manual Reverse pipetting (positive displacement pipetting)	

Automated	
AutoPrep reverse pipetting	
BDS (SP-1)	
BC	

Are your pipettes regularly calibrated?	
Is so, how often:	

Dual Platform:	
Haematology analysis:	
Haematology analyser used	
White cell count	x 10 ⁹ /l
Counting reference	
WCC	
Absolute Lymphocyte Count (ALC). (Refer to cover page)	

Flow cytometer differential:	
% Granulocytes	
% Lymphocytes	
% Monocytes	

Flow cytometry gating strategy:	
Lymphocyte gating FS/ SS	
CD3/4/8	
CD45 bright (Lymphs)	
abbreviated version of guideline CD3/4/8/45	
CD45 bright (Lymphs)	
3 or 4 colour panel (1998/ 2002 CDC/ NIH guidelines)	
CD14/ CD45 2 colour panel	
(6 tube 1992 CDC/ NIH guideline)	
PLG CD4 Total CD45	

APPENDIX B9

IMPORTANT: ALL PLG USERS

Please submit the BCXL raw data with results

RESULTS:
Predicate method = normal method used in your laboratory.
PLG method: If PLG method is predicate then write results in PLG row.
If both methods are is use, then write all results in the relevant rows.

Absolute counts			
Sample	CD4 T cells (Cells /µL)	CD8 T cells (Cells /µL)	CD3+ Total (Cells /µL)
Predicate QC 022			
Predicate QC 023			
PLG QC 022			
PLG QC 023			

Lymphocyte percentages			
Sample	CD4 T cells (Cells /µL)	CD8 T cells (Cells /µL)	CD3+ Total (Cells /µL)
Predicate QC 022			
Predicate QC 023			
PLG QC 022			
PLG QC 023			

Flow cytometer used:

BDS FACScan	
BDS FACSCalibur	
BDS FACSCount	
BC FC 500	
BC Elite	
BC XL	
Partec	
Ortho Cyturon	
Other:	

Red cell lysis:

Lysed – No wash	
Separated cells (eg FICOLL)	
Lysed and washed	
No wash – No lyse	

To be filled in by new participants

Maintenance:	
Are your instruments regularly maintained?	
Is so, how often:	
Are your instruments regularly serviced?	
Is so, how often:	

Routine laboratory specimens:	
How many CD4 specimens does your laboratory generate monthly? (approx)	
At the time of joining AFREQAS	
At the current date	

Comments:	

Suggestions:	

Date:	Signature:
Name (Please print):	

Thank you for your participation.
Professor D.K Glencross and Mrs H. Aggett.
CD4 AFREQAS



CD4+ Lymphocyte Proficiency Programme



Results of Survey number 4 of 2008 (QC026) Trial 31

Test Mean

Date of Report: 22 August 2008

LABORATORY: FLOWCYTOMETRY MED SCHOOL RD HAZEL PLG-SP

REGION: CD4 REFERENCE LABORATORY

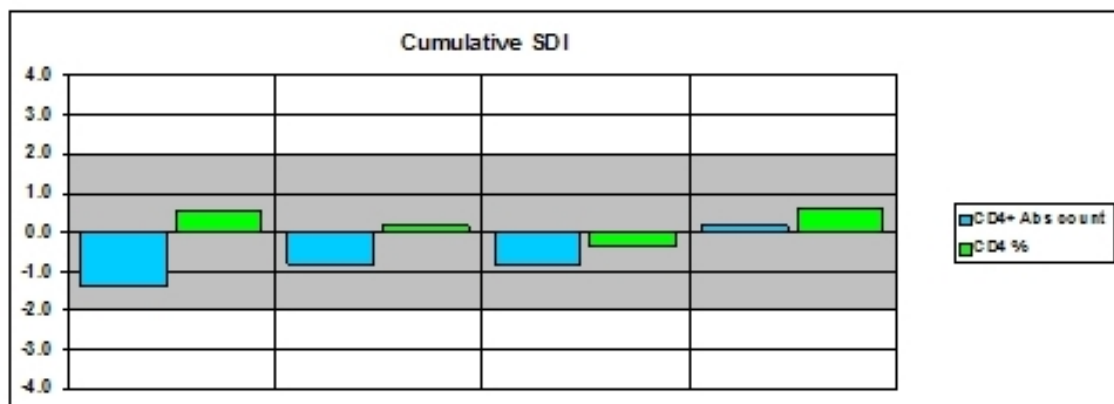
PROTOCOL: Lysed no Wash

INSTRUMENT: BCKL

Mab PANEL:

TUBE	Antibody 1	FL1	Antibody 2	FL2	Antibody 3	FL3	Antibody 4	FL4
1	CD45	FL1C	CD4	PE				

TEST	RESULTS	RESIDUAL	SDI	>+/- 2.0	GLOBAL ALL TECHNOLOGIES				
					METHOD	No.	MEAN	SD	CV
1) CD4+ Lymphocyte Absolute count	752.00	12.64	0.17		ALL	235	739.355	74.033	10.01
4) CD4 % of Lymphocytes	51.10	1.57	0.60		ALL	184	49.530	2.898	5.25



End Of Report

Permission to quote Professor C Larsen's paper:

APPENDIX C

Summary of problems and strategies for accessible support in the least developed countries

Hazel Aggett

From: Debbie Glencross [debbie@roussos.co.za]
Sent: 12 November 2008 07:18
To: Hazel Aggett
Subject: FW: permission

Associate Professor Deborah Glencross
Department of Molecular Medicine and Haematology Faculty of Health Sciences University of
the Witwatersrand Johannesburg
Email: debbie.glencross@nhls.ac.za
Tel: +27 11 489 8540

P Before printing, think about the environment

-----Original Message-----

From: Christoph H. Larsen [mailto:christoph.larsen@synaling.com]
Sent: 12 November 2008 04:24
To: Debbie Glencross
Subject: Re: permission

Dear Debbie,

Great to hear from you! Sure enough, you get my full and whole-hearted permission...
apart from the fact that this stuff has been published, and hence available for
digestion, quotation, whatever!

If you or your student have any queries, let me know. Things are constantly changing, so
do not hesitate to give him or her my contact details.

Good luck, and hopefully till soon!

Warmest regards,
Chris

Quoting Debbie Glencross <debbie@roussos.co.za>:

> Hello Christoph.
>
> Hope this email finds you well.
>
> I have asked one of my students, whose project it was to analyse data
> from the CD4 AFREQAS programme, to summarise the issues you addressed
> in your Cytometry into a table to use in her thesis. Obviously, I will
> only allow her to do this if she has your permission to do so (with
> full acknowledgement to you). She will use this table as part of her
> discussion addressing logistical and other issues around CD4 delivery
> of services in Africa.
>
> May we have your official permission please.
>
> Thanks much
>
> Regards
>
> Debbie
>
>
> Associate Professor Deborah Glencross
>
> Department of Molecular Medicine and Haematology
>
> Faculty of Health Sciences
>
> University of the Witwatersrand

APPENDIX C1

Summary of problems and strategies for accessible support in the least developed countries
(Extracted with permission from Christoph H Larsen. The fragile environments of inexpensive CD4+T-cell enumeration in the least developed countries: Strategies for accessible support. Cytometry Part B Clin Cytrom 2008; 74B:S107-S116).

	Problems encountered	Strategies and solutions
THE CHAOTIC LANDSCAPE OF AID		
	Health care remains weak due to chronic under funding of their primary health systems. Multiplicity of donors has created parallel infrastructures that are difficult to manage and undermine the responsibilities of public health services. Compromised the sustainability and implementation of ART and CD4 enumeration. Internal and external brain drain. Lack of funds for modern technology, maintenance and human resource development.	Need for more efficient operations, where reductions in unit costs are associated with increased numbers of tests and higher profits. Simplified technology with increased reliability with affordable laboratory involvement.
HARDWARE ISSUES		
Procurement	The procurement of high value equipment is guided by non-technical interests with mixed results. No ascertainable information available on novel instrumentation on performance, reliability and field installation requirements to obtain optimal results.	Instrument manufactures to streamline production. Simpler flow cytometers with smaller, cheaper and less power hungry lasers. Reduce pipetting steps as pipettes require regular calibrations and most sites do not have analytical balances.
Instrument support	Most installations operate without service contracts or protective shields after the one year warranty is up. Sustainability of equipment is under threat.	Rental agreements linked to service support. Modular bay design with pluggable tubing and wiring that can be exchanged in the case of malfunction could avoid costly shipping of entire pieces of equipment.
Enviromental factors	Dust exerts a detrimental effect on equipment. Need costly air-conditioning. Refrigeration needs electricity or fuel supplies. Unreliable power supplies causes damage to equipment. Power variations cause secondary damage to equipment and loss of production hours. Knocks sustained during instrument transport over poorly developed infrastructures causing misalignments of lasers.	Dust proof casing to reduce the number of dust-related incidents and service visits. Require a fast switching power supply with a range of 70-280 V Assisted with a professional surge protector. This setup is capable of providing adequate power conditioning for a wide range of environments including erratic mains, solar and battery/inverter combination supply settings.
POST-SALES ISSUES		
Reagent supplies	Poorly developed communication, transport and customs clearance infrastructure. Accounting procedures are restrictive. Advanced payment requirements.	Reagent rental agreements. A pay-as-you-go pricing concept absorbs the total cost of ownership, including training, maintenance, repairs and reagent shipping.

	Shipping and custom clearance is slow. Ordered from outside the end-users country.	
	Relatively short shelf life. Shipped reagents are time consuming. Perishable supplies require a constant cold chain with extra charges incurred. Expired reagents: the end-user incurs the cost if there is no contract agreement.	Long life reagents are required. Lyophilised reagents tend to have extended shelf life. Reagents should be heat stable and not require a cold chain facility. Benefits of less frequent ordering.
Service visits	Simple repairs may be done in one visit. In more severe cases a second visit is required after ordering spare parts. Laboratory staff tends to have problems mobilizing funds, followed by pre-payment causing long down-time. Shipping and customs clearance create further delays.	Simpler flow cytometers designed as desktop units with less need for laser adjustments. Remote instrument diagnostics and trouble-shooting technological innovations have rendered internet-based, a realistic and cost-efficient option.
Corporate service management	Main focus is on nurturing resource-rich environments. The end-users in poor settings are commonly serviced by stripped down versions of support systems.	Web-based customer-relationship management and enterprise resource planning software is expected to ease the existing complex communication and logistic issues. A public-private partnership is proposed that involve government, manufactures and local distributors with field application specialists.
Human resources	HIV/AIDS is taking its toll among health workers resulting in increased staff turnover. Poor staff retention in dysfunctional working environments, with inadequate support, missed salaries and cursory attention to staff development. Resignation of senior staff as no successors are trained even in basic maintenance procedures.	The health care workforce needs to be trained and supported to be committed.

SUPPLEMENTARY DATA

APPENDIX D

Longitudinal performance of individual laboratories

APPENDIX D1

Longitudinal performance was graphically represented by plotting sequential SDI results from a specific participating laboratory on circular (radar) plots. This would reflect the accuracy and precision of testing over time. These radar plots demonstrated when a particular laboratory joined the CD4 AFREQAS scheme, instances when there were outlying results or where there were no submission of results, showed when interventions improved performance, as well as overall accuracy and precision.

Performance with change of methodology could also be monitored. The selection of laboratories is representative of the performance across many participants which were specially chosen to represent either ideal performance or typical problems encountered through participation.

BDS FACSCount™ user precision

Across the twenty trials the BDS FACSCount™ users showed consistently good performance and this is shown on the graphical radar SDI performance of representative participants (Figure 3.34, Table 3.18). The minor problems that occurred were easily rectified with the help and advice given to the participants.

The performance of laboratories A, B and C is typical of the general performance of BDS FACSCount™ users on CD4 AFREQAS, showing consistently good performance across all trials. Laboratory D enrolled on the programme from Trial 2 and poor performance was reflected in an outlying result with an SDI exceeding 3.0. Consequently after corrective feedback and advice, the BDS FACSCount™ instrument in this laboratory was serviced and this participant became a consistent good performer for all subsequent trials. Laboratory E had one outlying result and overall a consistent performer. Laboratory F did not submit a result due to an unfortunate incident which occurred at their customs office containing the shipped samples. The parcel was destroyed by

custom officials as a 9/11 security procedure. There were two major problems that occurred with two other laboratories. Laboratory G joined the programme from Trial 6 showed consistent excellent results across nine trials, reflecting general performance of BDS FACSCount™ users. However, in the subsequent trials (16-20) this participant could not send in results due to problems with the instrument and a 6 month wait for repairs. Laboratory H joined the programme from Trial 6 and for four trials generated excellent results. However, in the next four trials the poor performance generated outlying SDI values >3.0. Initially, quality had been failing on this instrument and after numerous suggestions the problem was still not solved. This participant decided to change methodology to BDS TruCOUNT™/MultiSET™ on FACSCalibur instrument. Unfortunately, despite changing methodology, there was no improvement in the laboratory's overall performance.

BDS FACSCount™ users A-G

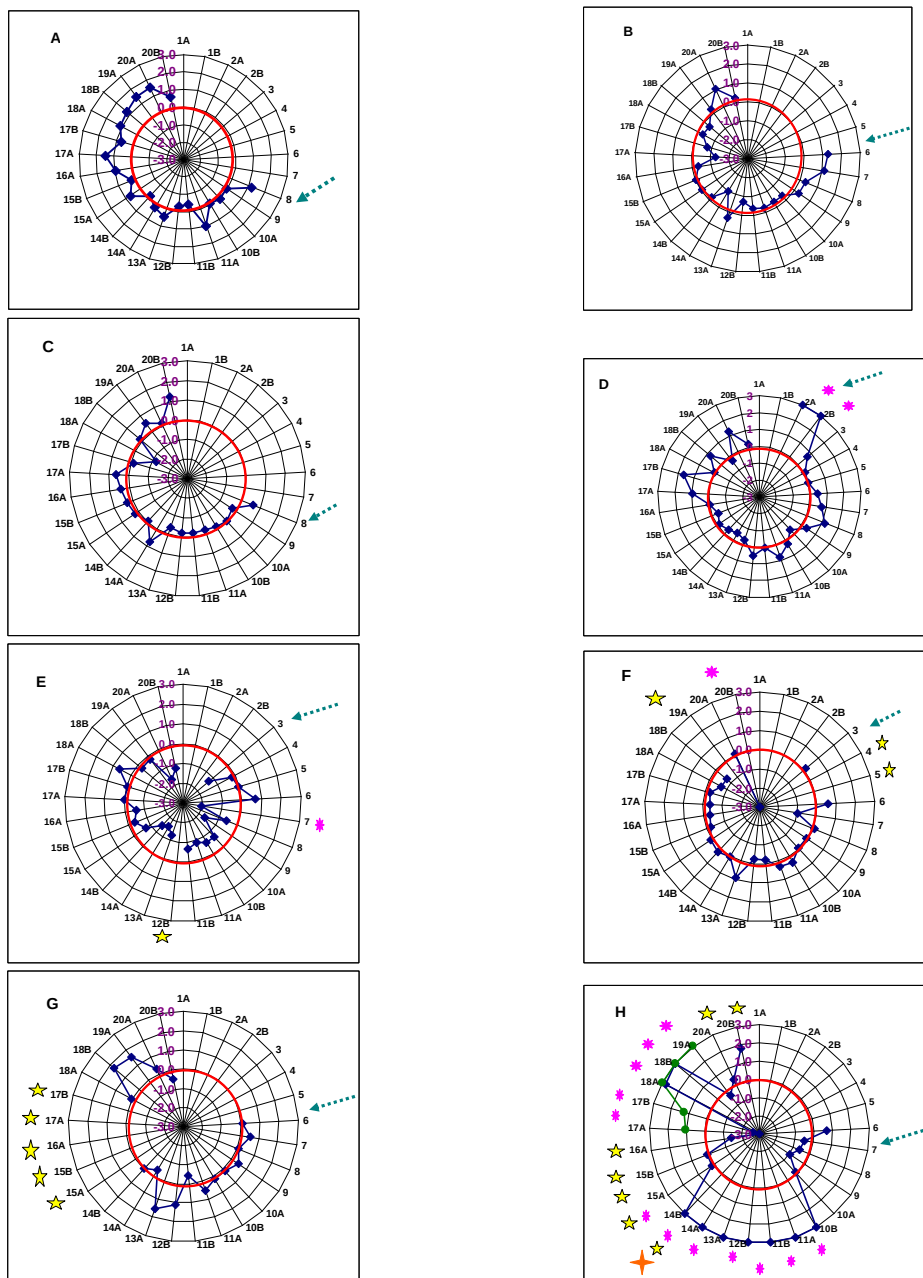


Figure 3.34: Representative selection of laboratories using BDS FACSCount™ methodology (A-H). BDS FACSCount™ is unable to generate CD4%Ly values and only abs counts with respect to submission is shown. The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green ♦) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. ♦ Represents outliers, while ★ represents non-submission for a particular trial ♦ Represents change of methodology

BDS TruCOUNT™/MultiSET™ users

Across the twenty trials the BDS TC/MS users showed poorer performance than BDS FACSCount™ users and this is shown on the graphical radar SDI performance of representative participants (Figure 3.35 3.36, Table 3.18)

Performance (precision) of laboratories using CD45 bright gating strategy

Across the twenty trials the performance of the users using CD45 bright gating showed a wider variation of submitted results. This is shown on the graphical radar SDI performance of representative participants (Figure 3.35 A-H) (Table 3.18). The irregular patterns noted are representative of the poorer precision and accuracy of some of these users, and this would be mainly attributed to poor understanding and application of the automated algorithm software of manually gating.

The performance of Laboratory A is typical of the performance of a laboratory using CD45 bright gating. Laboratory B showed adequate accuracy but the precision was erratic across all trials. Laboratory C enrolled on the programme from Trial 8 and for six trials used SP PLG CD4 method and performed well. From Trial 14 onwards this site changed to CD45 bright gating because this laboratory received an excellent instrument and reagent deal from the suppliers. This site was a consistent performer, having mastered a change of methodologies very well. Laboratory D enrolled on the programme from Trial 9 and for three trials did not submit results. No explanation was given. Subsequently, showed adequate accuracy but the precision was erratic across all trials. The poor performance of Laboratory E was reflected by the number of non submission of results and outlying results. Laboratory F, enrolled on the programme from Trial 3 and on four different trials the laboratory generated outlying results with SDI values of >3.0. On trial 16 this participant did not send in a result. In consultation with this participant it was revealed they had their FACSCalibur serviced and the staff received technical training. Reflective of training, this site became a consistent good performer for all subsequent trials. Laboratory G showed poor precision on the abs

counts and better precision for CD4%Ly. Laboratory H joined the programme from Trial 6 where performance was adequate. However, from Trial 10 to Trial 13 this laboratory's performance (SDI values of >3.0) deteriorated. This participant decided to change methodology from BDS FACSCount™ to BDS TruCOUNT™/MultiSET™, from Trial 14 their performance did not improve. In communication with this site, it transpired that they had experienced problems in getting additional training on their instrument and software applications and they were also concerned about their pipettes. Advice and help was given on pipetting techniques and validation of pipettes. Subsequently, the site received additional training from BDS on their instrument and software applications. Since Trial 20 their performance has improved dramatically reflecting the impact of appropriate interventions.

BDS TC/MS CD45 bright gating, A-G

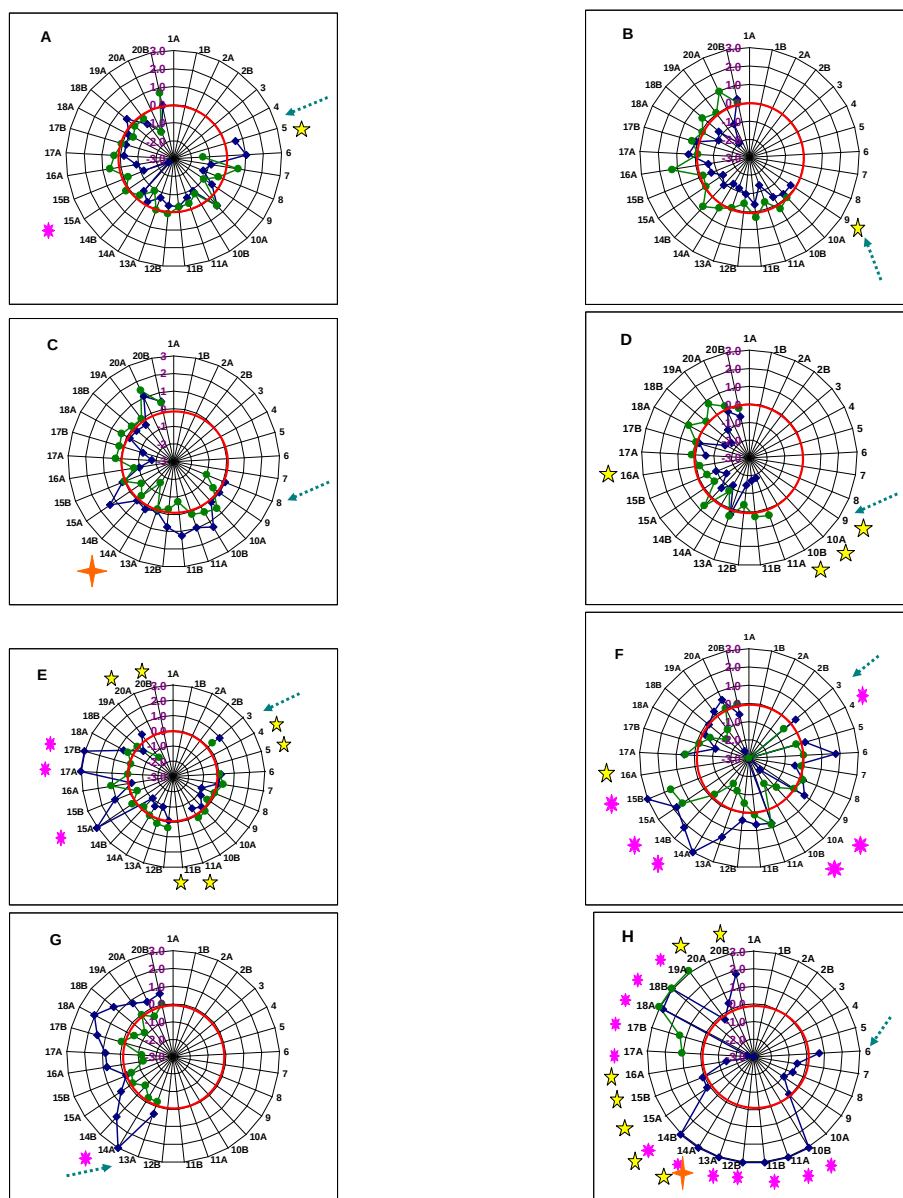


Figure 3.35: Representative selection of laboratories using BDS TC/MS CD45 bright gating methodology (A-H).

The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green ●) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. *Represents outliers, while ★ represents non-submission for a particular trial ♦ Represents change of methodology

Performance (precision) of laboratories using primary CD3 gating strategy

Across the twenty trials laboratories using primary CD3 gating showed a wide variation of submitted results. This is shown on the graphical radar SDI performance of representative participants (Figure 3.36 A-H) (Table 3.18). The poor performance was reflected by a high number of outlying results (>3.0) with an outlier rate of 21.6%. The irregular patterns noted are representative of the poorer precision and accuracy of some of these users, and this would be mainly attributed to poor understanding and application of the automated algorithm software of manually gating. Primary CD3 gating were prone to incorrectly report lymphocyte percentages, CD4% of T cells (as a consequence of Primary CD3 gating) was frequently incorrectly reported instead of the required values.

Laboratory A performed better than Laboratory B. All the CD4 absolute counts were within the 2 SD range. They incorrectly reported CD4% of T-cell values and as a consequence had three outlying results. Laboratory B showed the same problems experienced by Laboratory's A. Another laboratory in South Africa, Laboratory C had endless logistical and staff problems from Trial 3 to Trial 12, having experienced a high turnover rate of technologist. From Trial 13 new management was put into place and the laboratory took up the suggestion to employ skilled personnel, training as well as implementing additional staff motivation programmes. From Trial 13 to Trial 20 the laboratory's performance had substantially improved reflecting the impact of training. Laboratory D had been given initial training on the FACSCalibur by the supplier BDS. No results were submitted from Trial 16 to Trial 18 as the instrument required a new part. The instrument was subsequently repaired by the BDS service engineer. After being repaired, the laboratory still sent in results over the 2 SD limits for both abs counts and CD4%Ly. To-date, this site changed gating methodology to CD45 bright gating and their performance had improved (data not shown). Laboratory E, incorrectly reported CD4%Ly from Trial 3 to Trial 17 by reporting CD4% of T cells (as a consequence of Primary CD3 gating). The poor performance was reflected by this high number of outlying results (>3.0). However, for abs counts, all their results submitted fell within the 2 SD

range. This site did do all the necessary maintenance, pipette validation and regular servicing of the FACSCalibur. It was also recommended by Beckton Dickinson, the supplier, to change to CD45 bright gating as this method is more reproducible. The site reported that poor performance and problems with using primary CD3 gating was also reflected in their under performance on the UK NEQAS Scheme. The laboratory did take the advice and change methodologies to the recommended four colour CD45 bright gating. From However from Trial 19, and on all subsequent trials (data not shown) this laboratory did became a good and consistent performer.

Occasionally participants experience problems with both abs counts and CD4%Ly reporting Laboratory F joined the CD4 AFREQAS programme from Trial 10 and from this trial to Trial 20 demonstrated poor performance with respect to both abs counts and CD4%Ly results. This was unfortunately a high through-put laboratory generating CD4 results for patients on ARV treatment. It was suggested that they change gating methodology to the four colour CD45 bright gating strategy. However the laboratory indicated that they thought that the CD4 AFREQAS material was the problem and did not respond to corrective actions. This laboratory was closed down by their management after Trial 20. Laboratory G is an example of a site that needed manufacturer input and training. From registration on Trial 10, and all subsequent trial, this site had problems with abs counts and CD4%Ly, as well as non submission of results. This site was a poor performing site and only after Trial 20 did this site change to CD45 bright gating strategy and improved on their performance (data not shown). Laboratory H is an example how continued ongoing corrective input and switching to CD45 bright gating can improve performance of participating laboratory

BDS TC/MS Primary CD3 gating, A-G

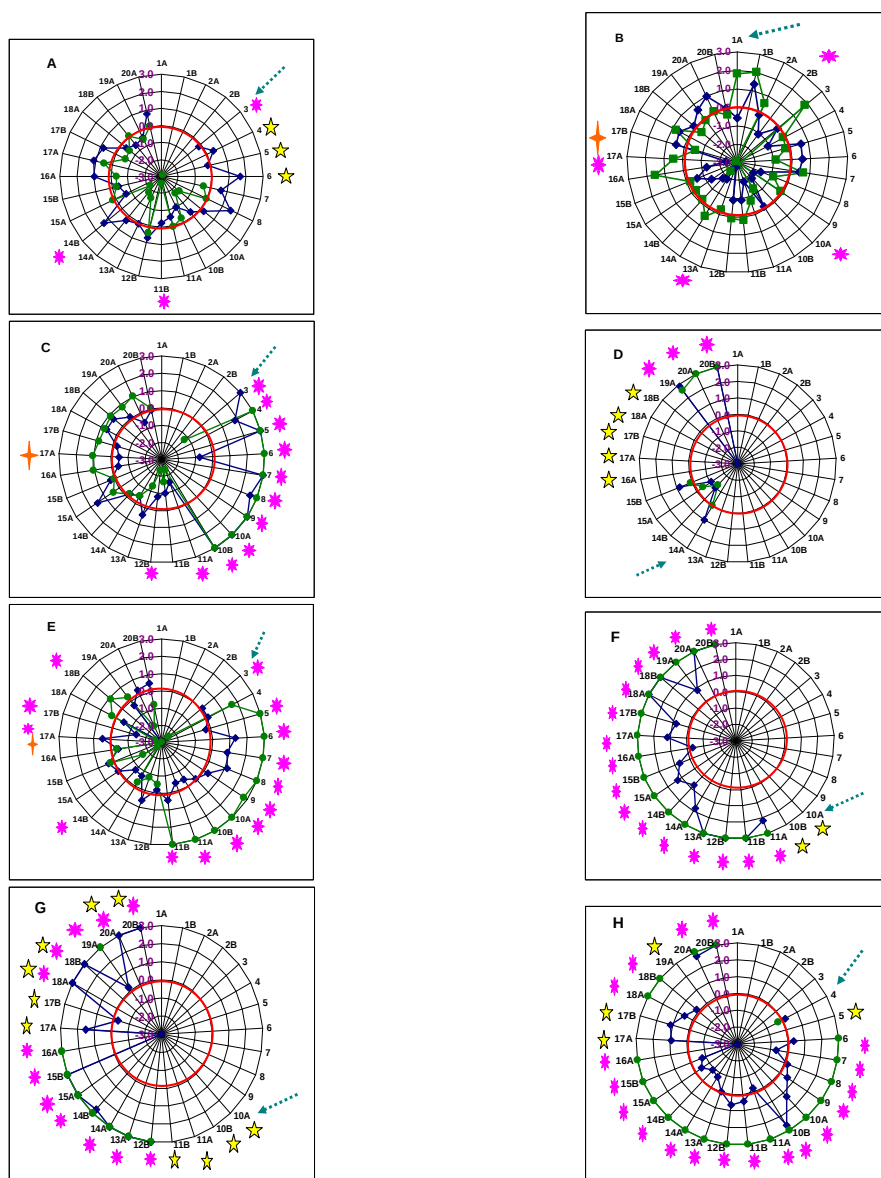


Figure 3.36: Representative selection of laboratories using Primary CD3 gating methodology (A-H). The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green •) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. * Represents outliers, while ☆ represents non-submission for a particular trial ♦ Represents change of methodology

SP PLG-CD4 user precision

Across the twenty trials the performance of the participants using SP PLG-CD4 users showed consistently good performance and this is shown on the graphical radar SDI performance of the representative participants (Figure 3.37 A-H, Table 3.18). SP PLG-CD4 methodology generated outlying results (SDI values of >3.0) for abs counts of 5.4% and CD4%Ly of 3.1%. The minor problems that occurred included manual gating, incorrect reporting of CD4% of WBC and transcription errors. These were easily rectified with the help and advice given to the participants.

Laboratory A is representative of a consistently good performance across twenty trials. Laboratory B joined the programme from Trial 1 and for five trials demonstrated excellent performance using DP PLG CD4 methodology. From Trial 6 this site changed to single platform and remained a consistently good performing laboratory, demonstrating the ease of transition of a laboratory using PLG from dual platform to single platform methodology. Laboratory C is representative of a consistently good performance across twenty trials. Laboratory D is representative of a consistently good performance across twenty trials. Laboratory E had an outlier on their first trial, Trial 6. It was noted that the pipettes were never calibrated and were subsequently calibrated and from Trial 7, the results were well within the 2 SD limits. Participant F had one outlier while they participated on the AFREQAS programme, and this was due to incorrect reporting %CD4 of white cells instead of reporting a %CD4Ly values. Typically, this type of error did not reoccur after corrective action intervention. Laboratory G and H each had a single outlying result in Trial 1 due to submitting low absolute CD4 counts on “normal” value material. This error was due to incorrect gating protocol. After corrective action, these two laboratories remained consistent good performers. What was especially noted with SP PLG CD4 method was the consistent reliable precision of CD4%Ly values reported by these users. With the use of CD45 bright and side scatter PLG protocol, monocytes are excluded from the CD4 lymphocyte population and excellent precision reported.

All SP PLG-CD4

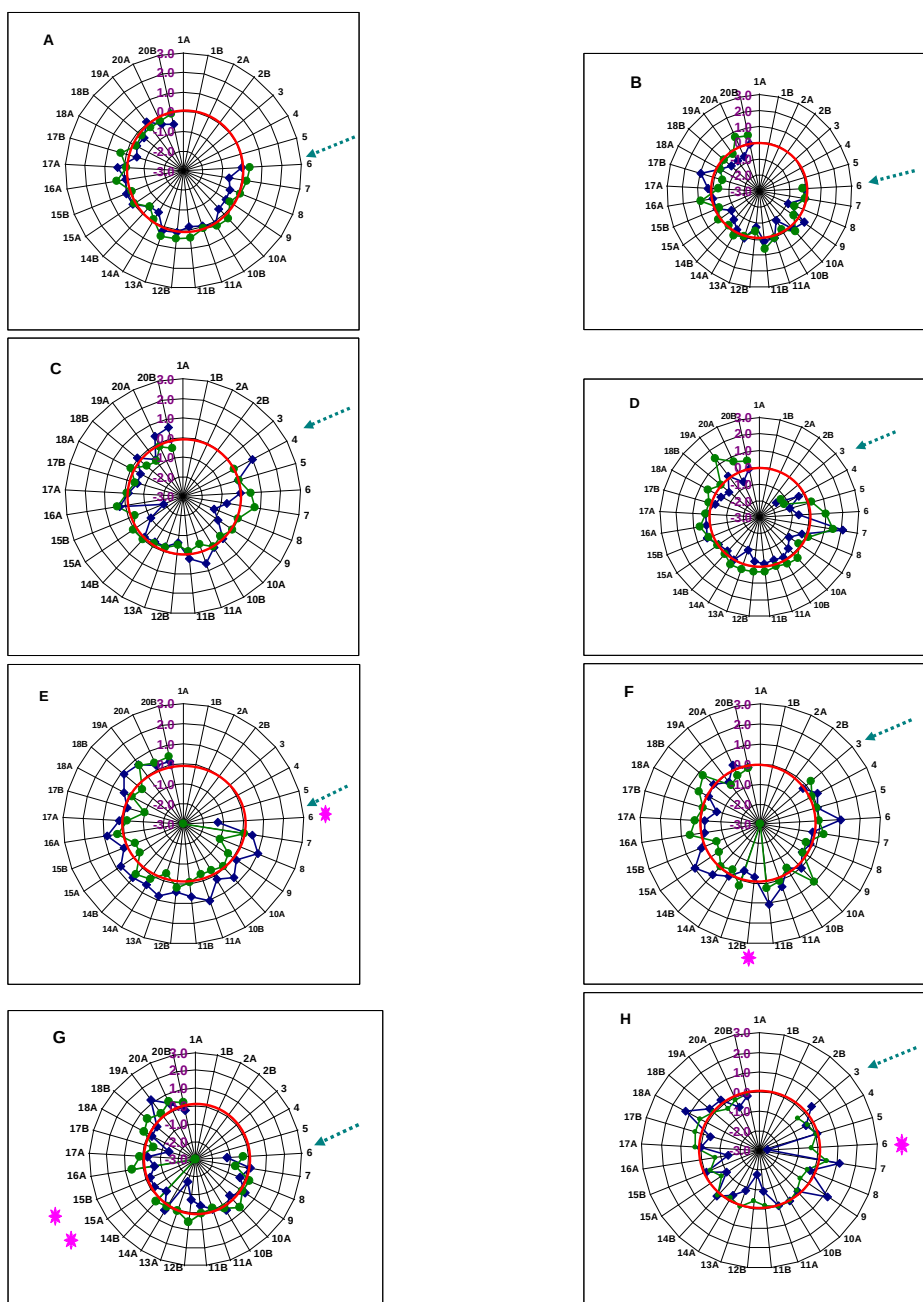


Figure 3.37 Representative selections of laboratories using SP PLG-CD4 methodology (A-H).

The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green ★) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. ★ Represents outliers, while ☆ represents non-submission for a particular trial ♦ Represents change of methodology

Partec® user precision

Across the twenty trials the performance of the participants using Partec® showed a wider variation of submitted results in which a vast majority of results fell outside the 2 SD acceptable range. The poor performance was reflected by the high number of outlying results of abs counts (31.9%).

This is shown on the graphical radar SDI performance of the representative participants (Figure 3.38 A-H, Table 3.18). The irregular patterns that are shown demonstrate poorer precision and accuracy of individual users. The main problem observed with these users was the lack of training and maintenance of instruments at these sites. There was an overall average rate of 27.4% noted across the 20 trials of non submission of results due to down time of these instruments.

Overall, the participants using this volumetric method did not perform well. A reference laboratory from Germany, Laboratory A, supported two Mission Hospitals in Africa. The performance of this laboratory was good, suggesting that there is no obvious incompatibility of the performance with Partec® instruments. The Partec® users did get initial training on the instruments when they were placed in the laboratories. However follow-up training and service delivery was poor as evidenced by the performance of Participants B and C (Figure 3.8). Participant B, their first submitted result on joining was within the 2 SD limits but on subsequent trials, Trial 10 to Trial 20, this laboratory had problems with the Partec® instrument and the submitted results fell outside the 2 SD limits. On Trial 19 the trimmed SDI value was 23.1. With ongoing corrective action feedback, Participant B changed their method to the BDS FACSCount™ and has since shown to be an excellent participant on the CD4 AFREQAS scheme. Laboratory C is a participant who struggled to get maintenance and repairs for the instrument after it was placed in their laboratory. The suppliers did give the laboratory training on the instrument when they placed the instrument in the laboratory. At one stage the instrument was out of order for four months and the laboratory could not generate CD4 counts for patients. Medecins Sans Frontiers (MSF) supported laboratories in southern rural districts of Thyolo and Chiradzulu and the central district of Dowa in Malawi. They placed Partec® Cyflow

instruments into these sites but before getting them installed asked the CD4 AFREQAS programme about the instrument. They took it upon themselves to get intensive training to all their supported sites. Laboratory D is one of the sites and has been a consistent good performer since joining AFREQAS from Trial 18 and all subsequent trials (data not shown). Once again, emphasizing the importance of instrument maintenance, training and manufacturer support.

Partec® users A-H

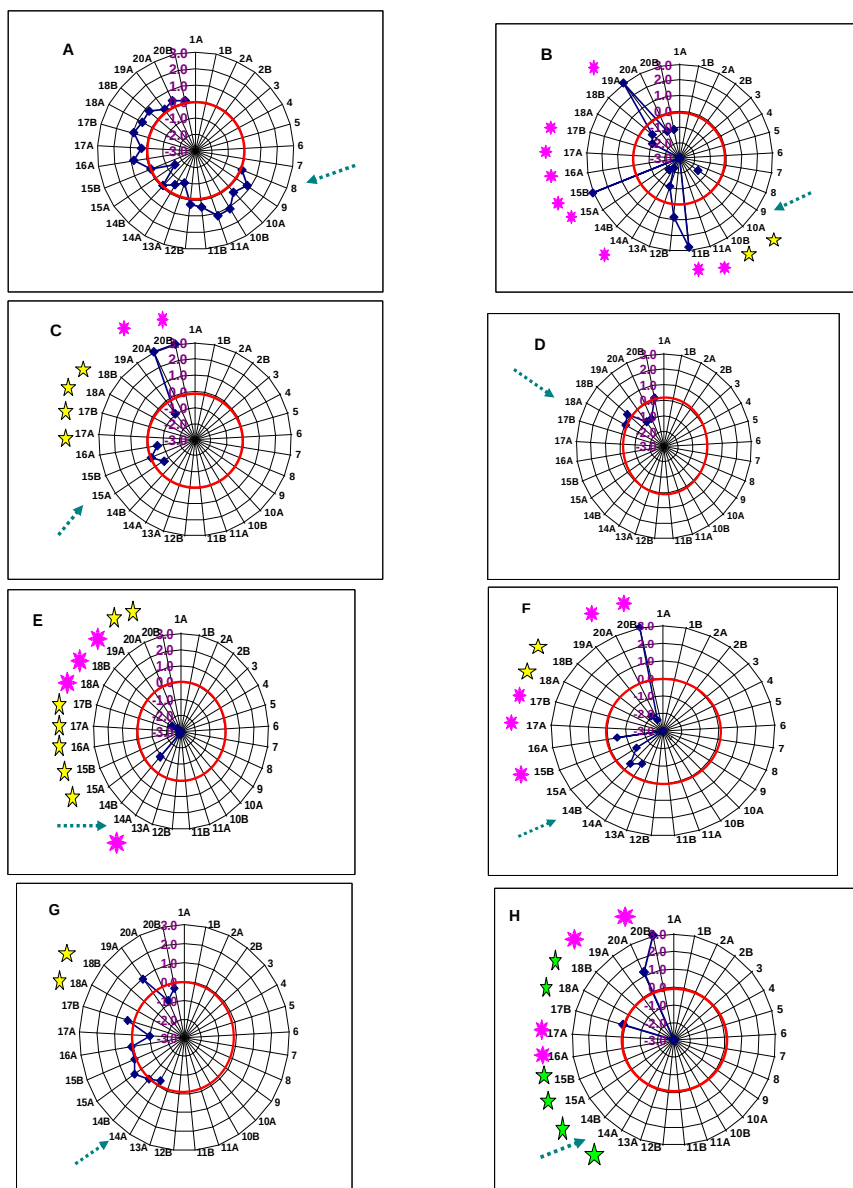


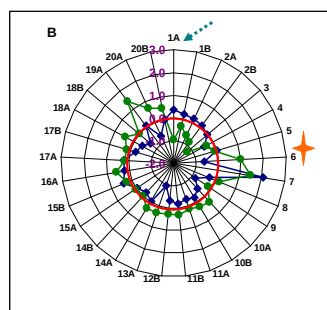
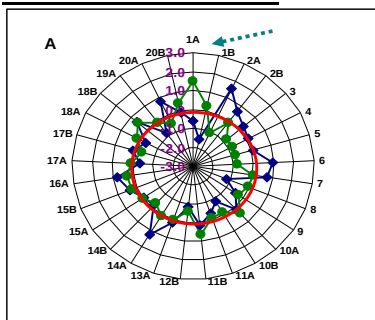
Figure 3.38: Representative selections of laboratories using Partec® methodology (A-H). Partec® methodology is unable to generate CD4%Ly values and only abs counts with respect to submission is shown. The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green ★) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. ★ Represents outliers, while ★ represents non-submission for a particular trial. ♦ Represents change of methodology.

Dual platform user precision

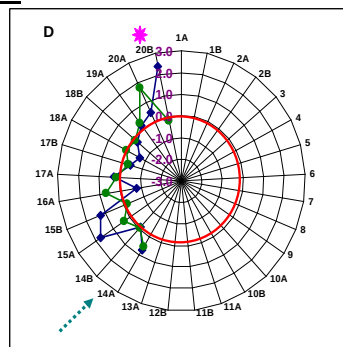
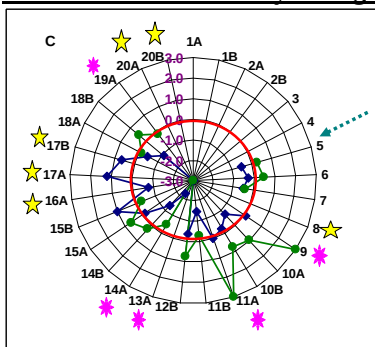
Participant C from Zimbabwe enrolled on the programme from Trial 5 and from Trial 8 the laboratory had outliers and no submission of results. This is a laboratory that had no technical and financial support and used expired reagents. Both the Haematology analyzer and FACSCalibur could not get serviced due to the financial constraints of the laboratory. However, Participant D from South Africa had very good technical support and training from the respective suppliers and showed to be a good performing participant.

Participants E and F (figure 3.39) enrolled on the programme using dual platform primary CD3 gating and changed to single platform four colour CD45 *bright* gating strategies. Participant E was a poor performer even after changing methods. This laboratory received training from the suppliers but could not grasp the technology. They subsequently closed down due to financial constraints. Participant F is an example of continued ongoing corrective input to bring performance of laboratory using Dual platform Primary CD3 gating in line with expected performance of TruCOUNT™/MultiSET™ CD45 *bright* gated CD3/CD4/CD8.

DP PLG-CD4 users A-B

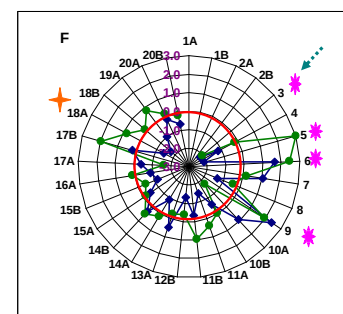
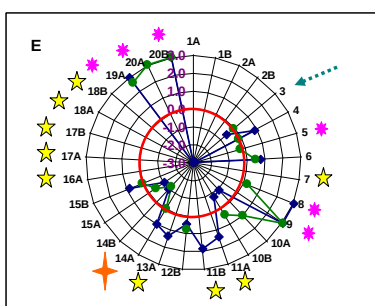


DP MultiSET™ Primary CD3 gating with CD4/8 users C-D



DP BDS MultiSET™ Primary CD3 gating with/4/8 changed to single platform Trucount™ evaluation

E-F



DP TetraONE™ CD45 bright gating CD3/4/8 users G-H

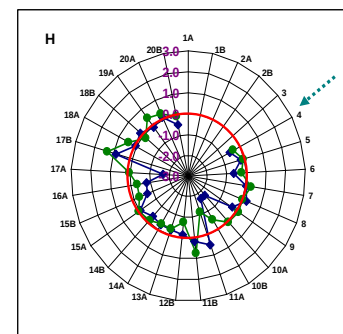
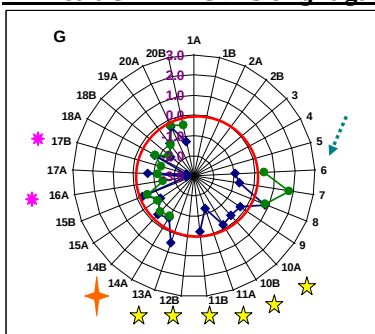


Figure 3.39: Longitudinal SDI follow-up performance of a selection of laboratories using dual platform systems

The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green ★) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. ★ Represents outliers, while ☆ represents non-submission for a particular trial —◆— Represents change of methodology

Beckman Coulter system TetraONE™ and FlowCount™ user precision

Across the twenty trials the performance of the laboratories showed consistently good performance using TetraONE™ FlowCount™ with CD45 bright gating. This is shown on the graphical radar SDI performance of representative participants (Figure 3.40 A-H). There were no problems encountered in laboratories using this technology on the CD4 AFREQAS programme and no outlying results were encountered across twenty trials. However, it is important to mention that all the sites were within South Africa and had direct access to training within the National Health Laboratory Service and access to training from Beckman Coulter South Africa. Once again this emphasises the importance of the role of training and manufacturer support in the delivery of quality CD4 testing.

All Laboratories (A-H) showed consistently good performance. Laboratory F joined AFREQAS from Trial 3 and changed technology to SP PLG CD4 from Trial 10. This was due to the laboratory's participation in an ARV programme in Kwa -Zulu Natal in which SP PLG CD4 offered the more cost effective method in line with the NHLS National ARV programme. Participants G and H used this technology for a clinical trial study which was implemented from Trial 17 and both abs counts and CD4%Ly showed very good performance for all four trials and subsequent trials (data not shown).

Beckman Coulter system TetraONE™ and FlowCount™ A-H

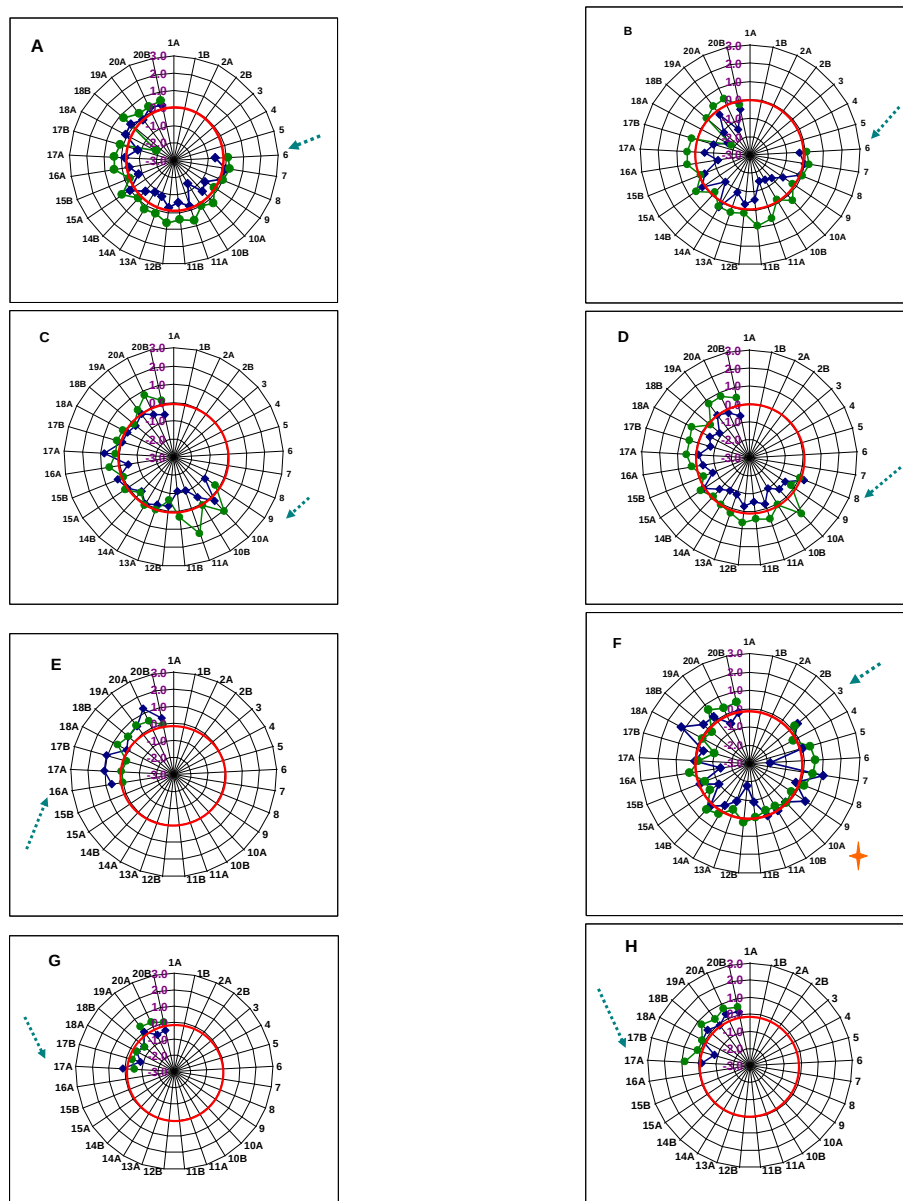


Figure 3.40: Representative selection of laboratories using Beckman Coulter SP TetraONE™ CD45 bright gating (A-H).

The X-axis is from -3 to +3 and trial numbers are indicated from 1A to 20B on the Y-axis. SDI values are plotted for both the CD4 abs counts (Blue ♦) and CD4%Ly values (Green •) for a particular laboratory. The blue arrow indicates when the laboratory enrolled on the CD4 AFREQAS scheme. The target SDI of 0 (red line) is indicated for comparison of data to the expected target. * Represents outliers, while ★ represents non-submission for a particular trial ➡★ Represents change of methodology

SUPPLEMENTARY DATA

APPENDIX D 2

Table 3.18: Summary of problems identified and corrective actions for CD4 AFREQAS participants

Figure	Trial number	Methodology	Problems identified	Corrective action interventions
Within trial precision of African laboratory networks				
3.20	20 (Number A)	<u>South Africa (NHLS)</u> SP PLG CD4	Trial 20 isolated outlier. Transcription error.	Incorrect reporting %CD4 of WCC. Corrected in subsequent trials (data not shown).
	6 (Number C)	<u>South African (NHLS)</u> SP PLG CD4	First trial low results submitted.	Corrective solutions offered. FCR exercise done (Section 3.11). Subsequent results were within $\pm 2SDI$.
	18A (Number F)	<u>South African (NHLS)</u> SP PLG CD4	From trial 6 to 20 only one result out due to transcription error. Trial 10 no %CD4 of lymphocytes reported. Laboratory forgot to write in result.	Laboratory identified problem. Subsequent results were within $\pm 2SDI$.
3.21	4/10/14/15 (Number E)	<u>Botswanan (NGL)</u> BDS TC/MS CD45 bright gating	Low results submitted for four trials. Participant needs to acquire skills for manual gating.	Corrective solutions offered including, to receive technical training by BD on manual gating as protocol is automated.
	16/25/30 (Number E)	<u>Botswanan (NGL)</u> BDS TC/MS CD45 bright gating	Non submission of results as instrument out of order. Requested advice on improving results.	BD did training at laboratory. From Trial 17 results were within $\pm 2SDI$. Great improvement noted.
	15-20 (Number G)	<u>Botswanan (NGL)</u> BDS TC/MS CD45 bright gating	Non submissions and outlying results.	Corrective solutions offered including suggested software application and training by BD as well as pipette calibration and pipette technique training
	15-20 (Number H)	<u>Botswanan (NGL)</u> BDS TC/MS CD45 bright gating	Non submissions. FACSCalibur not working. Laboratory could not fix the problem	Suggested contacting BD.
3.22	(Number A-E)	<u>Namibian (NIP)</u> SP PLG CD4	Three of Namibia's laboratories had problems of non submission of results as well as low or high absolute counts.	Non submission of results was due to internal laboratory staff problems. Subsequently sorted out and results sent back in time for analysis. Wrong absolute counts were due to pipetting errors. Need to introduce monitoring of FCR. Namibia has as yet not done so.
3.23	14-15 (Number A)	<u>Lesotho (NGL)</u> BDS TC/MS CD45 bright gating	In two out of seven trials no results were submitted. Three trials results were $>\pm 2SDI$.	Corrective solutions offered including suggesting software application and training by BD as well as pipette calibration and pipette technique training.

	14-16 (Number B)	<u>Lesotho (NGL)</u> BDS TC/MS CD45 bright gating	Two out of seven trials no submission of results. Four trials results were $>\pm 2SDI$.	Corrective solutions offered including software application and training by BD, changing to CD45 bright gating as well as pipette calibration and pipette technique training. After Trial 20 this site did not improve.
	14-16/19/20 16/19/20 14-20 15-20 14-20 (Number C-h)	<u>Lesotho (NGL)</u> Partec instruments®	All laboratories using Partec® had problems with non submission and low absolute count results. Instruments frequently down with little support.	As a corrective solution, suggested to get basic maintenance and repair training on the Partec® as well as technical training. The same problems have continued after trial 20. Little improvement in submitted results for all sites.
3.24	20 (Number B)	<u>Zimbabwe (NGL)</u> BDS FACSCount™	One result with low absolute count.	To monitor BDS FACSCount™ as there was an isolated outlier. This site had continued procurement issues with many non submissions.
	(Number D)	<u>Zimbabwe (NGL)</u> BDS TC/MS CD45 bright gating	Low and high results submitted.	As a corrective solution, suggested software application and training by BD on manual gating to enable participant to override automated software as required. Erratic results and non submission of results continued post trial 20.
	(Number E)	<u>Zimbabwe (NGL)</u> Dual platform TC/MS CD45 bright gating	Low absolute count values submitted for all trials.	Corrective solutions offered including software application and training by BD as well as pipette calibration and pipette technique training. Haematology analyser also needed servicing. Problems in getting it serviced.
	(Number G)	<u>Zimbabwe (NGL)</u> Partec instruments®	Low absolute count values submitted.	Laboratory decided the instrument needed a service as the laboratory had inherited the instrument. However, could not get it serviced due to no support from Partec®
Within trial precision of grant supported laboratory networks				
3.26	10-12 (Number D)	<u>Grant supported network "A"</u> BDS FACSCount™	For the first three trials non submission of results. No reasons given for non submissions.	From subsequent trials all results were within $\pm 2SDI$. Laboratory performed very well on the AFREQAS programme.
	9-17 (Number E)	<u>Grant supported network "A"</u> BDS TC/MS CD45 bright gating	From Trial 9 -17 had major problems with the technology.	Did validation study to assist laboratory. See validation report (Section 3.11.2). Laboratory showed consistent good performance with BDS TC/MS CD45 bright gating and new method BDS FACSCount™
	9-20 (Number F)	<u>Grant supported network "A"</u> BDS TC/MS Primary CD3	Had problems of non submission of results and when results were	Corrective solutions offered including suggesting technical training by BD, changing to

		gating	submitted the performance was outside of 3SD. This laboratory required more training.	CD45 gating. Post Trial 20 this laboratory started to perform better. Results not shown.
3.27	10-12 (Number B)	<u>Grant supported network</u> "B" BDS FACSCount™	Initial three trials non submission of results. Laboratory staff was still in training when the laboratory registered on AFREQAS. Hence the delay in submission of results.	All subsequent trials results were within $\pm 2SDI$.
	10/19 (Number C)	<u>Grant supported network</u> "B" BDS FACSCount™	Two trials non submission of results. Not satisfied with results, did not send them in. All other trials results were within $\pm 2SDI$.	This laboratory did not require corrective intervention. Explained to this site that non submission of results was unacceptable on participation in an EQA scheme. Shows poor performance of laboratory.
	15-17 (Number D)	<u>Grant supported network</u> "B" BDS FACSCount™	Three trials non submission of results. Laboratory was not satisfied with instrument.	Instrument serviced after a long delay. After the service, all results were within $\pm 2SDI$.
General problems encountered in countries				
Senegal		<u>University laboratory</u> BDS FACSCount™	Customs security alert. USA 9/11 terrorist attack. No samples received.	No intervention required.
Tanzania		<u>Private laboratory</u> Guava PCA	Non submission of results for all trials. Laboratory discontinued their participation with CD4 AFREQAS.	Laboratory did not respond to corrective solutions offered.
Ivory Coast		<u>University R/D laboratory</u> BDS TC/MS CD45 bright gating	Initial non submission of results. Laboratory could not get parcel through customs. Custom service required AFREQAS Export Permit in French as well as English.	After numerous telephone calls to customs department and Home affairs in Ivory Coast a French export permit was granted to the CD4 AFREQAS programme. This site has since shown to be a consistent good performer. Very good results submitted using BDS TC/MS CD45 bright gating. They mastered this methodology.
Burundi		<u>University laboratory</u> DP BDS TC/MS Primary CD3 gating	Six submitted results over fifteen shipments received.	Major problems with custom clearance. CD4 AFREQAS could not help the laboratory to sort out the logistics involved. This is a continuing problem that the participant needs to sort out. From Trial 20, the custom clearance issue had not been resolved.
Angola		<u>Military Hospital Laboratory</u> DP BDS TC/MS Primary	One submission from sixteen trials. CD4 EQA material went	Government and political interference. The laboratory was given permission to receive the

	CD3 gating	through customs. Results could not be submitted.	CD4 EQA material by the Military department but was not allowed to submit the results to CD4 AFREQAS. The department would not give a reason for non submission. Subsequent to Trial 20 the laboratory discontinued participation.
Kenya	<u>Mission Hospital</u> BDS FACSCount™	Three trials non submission of results.	In consultation with the participant, the lack of power supply to the laboratory was the reason for non submission of results. No corrective intervention could be given. However, when results were received these were <2SD.
Ghana	<u>Private laboratory</u> BDS FACSCount™	Three trials non submission of results. This was due to problems with custom clearance.	AFREQAS addressed the problem with the relevant person at customs and the courier company. Subsequent shipments passed through customs smoothly. Subsequent results all within <2SD. However, CD4 results within the 2SD though.
Zambia	<u>Small District Hospital Laboratory</u> SP PLG CD4	Three trials non submission of results. Rats ate cables of phone and fax lines.	Cables had to be ordered from South Africa with further delays. No corrective intervention could be given.
	<u>Private laboratory</u> BDS TC/MS CD45 bright gating	Three trials non submission of results. The lack of power supply to the laboratory was the reason for non submission of results.	Laboratory installed back-up generator. Submitted results were erratic. As a corrective solution, suggested software application and training by BD on manual gating to enable participant to override automated software as required. Erratic results and non submission of results continued post trial 20.
	<u>Mission Hospital</u> Partec instruments®	One trial non submission of results. The donkey passed away. CD4 AFREQAS parcel could not get to remote Mission Hospital in time for submission of results.	A scooter has replaced the donkey. However, performance was marred by other factors including no support to service instrument. Waited for spare parts. This problem continued after Trial 20.
Botswana	<u>Private laboratory</u> BDS FACSCount™	Two trials non submission of results.	Cleaning staff signed for the two deliveries and placed the parcels in the broom cupboard and forgot about them. In-house problems have been sorted out. Subsequent results all within <2SD. Excellent performance shown.
	<u>Private laboratory</u> BDS FACSCount™	Four trials non submission of results.	AFREQAS explained the importance of participation.

		Laboratory did not think it was important to submit all results timeously.	Results for all subsequent trials were received on time. Very good performance shown.	
Longitudinal SDI performance of individual laboratories over time				
3.34	2A-2B (Number D)	BDS FACSCount™	High absolute counts. SDI>3.0	Instrument serviced. From Trial 3 performance was improved.
	19A (Number G)	BDS FACSCount™	One result SDI>-3.0 Isolated outlier.	No corrective action. Good performing laboratory.
	15A-17B (Number H)	BDS FACSCount™	Non submission of results for three trials. No support to service instrument. Waited for spare parts.	No corrective action required. From Trial 18 consistent good performing laboratory.
3.35	4/5/11/20 (Number E)	BDS TC/MS CD45 bright gating	Non submission of results for four trials and two trials had high absolute CD4 counts.	Suggested technical training by BD. Laboratory thanked AFREQAS for showing interest and would troubleshoot their problems. Performance improved
	14A-14B (Number F)	BDS TC/MS CD45 bright gating	High absolute results submitted.	Suggested instrument service and technical training by BD.
	16 (Number F)	BDS TC/MS CD45 bright gating	No results submitted. The laboratory could not fix instrument.	FACSCalibur service and repair advised. Results from Trial 17-20 within ±2SDI.
	10B-13 (Number H)	BDS FACSCount™	FACSCount generated high counts for three trials. Could not get the FACSCount to give correct control values.	Changed technology to TruCOUNT™/MultiSET™. Advised the participant to get technical training by BD.
	14-20 (Number H)	BDS TC/MS CD45 bright gating	Changed technology from FACSCount from Trial 14. Had problems from Trial 14-20.	Again suggested technical training by BD with pipette calibration and pipette technique training.
3.36	3/11B14B (Number A)	BDS TC/MS Primary CD3 gating	Lymphocyte % results low on three trials.	Laboratory did not respond to corrective solutions offered.
	2B/10A/13A	BDS TC/MS Primary	Four trials low	Suggested

	(Number B)	CD3 gating	results submitted.	calibrating pipettes or changing to CD45 bright gating. Calibrated pipettes. The laboratory monitored performance. Changed technology to CD45 bright gating Improved performance. Results within ± 2 SDI.
	3-12B (Number C)	BDS TC/MS Primary CD3 gating	For ten trials laboratory submitted high results. Endless logistical and staff problems. High staff turnover.	Suggested technical training by BD as well as pipette calibration and pipette technique training.
	13-20B (Number C)	BDS TC/MS Primary CD3 gating	From Trial 13 all subsequent results were within ± 2 SDI.	New management, new staff. Took up suggestion of training and motivating staff.
	16A-20B (Number D)	BDS TC/MS Primary CD3 gating	From Trial 16 no submitted results and SDI results $> \pm 3$. National logistical and financial difficulties.	Corrective solutions offered, suggested changing to CD45 bright gating. Trial 21 changed to CD45 bright gating and subsequent results were within ± 2 SDI (data not shown).
	5-20B (Number E)	BDS TC/MS Primary CD3 gating	From Trial 5 had problems with CD4%Ly.	Corrective solutions offered, suggested changing to CD45 bright gating. No action taken. Withdrew after Trial 20.
	3-17B (Number E)	BDS TC/MS Primary CD3 gating	From Trial 3 submitted results that were very high or extremely low. Also had problems with UKNEQAS programme.	Corrective solutions offered including changing to CD45 bright gating. Trial 18 changed to CD45 bright gating and subsequent results were within ± 2 SDI.
	10A-20B (Number F)	BDS TC/MS Primary CD3 gating	From joining AFREQAS very high results submitted.	Laboratory did not respond to corrective solutions offered.

			Laboratory concluded the CD4 EQA material was a problem.	Management stopped routine CD4 testing in this laboratory.
	10A-20B (Number G)	BDS TC/MS Primary CD3 gating	From joining AFREQAS had submitted very erratic results. Skilled staff shortages.	Multiple corrective solutions offered including changing to CD45 bright gating. For ten trials no intervention helped. Subsequently, changed to CD45 bright gating. Improved performance from trial 20 results not shown.
3.37	6 (Number E)	SP PLG CD4	First trial low results submitted.	Calibrated pipettes. Subsequent results were within ± 2 SDI.
	12B (Number F)	SP PLG CD4	From trial 3 to 20 only one result out. Isolated outlier. Transcription error.	Incorrect reporting %CD4 of WCC. Corrected in subsequent trials.
	15A-B (Number G)	SP PLG CD4	Low results submitted. SDI >- 3.0	Checked pipetting technique. Subsequent results were within ± 2 SDI.
	6 (Number H)	SP PLG CD4	Low absolute count submitted. SDI >- 2.0	Checked pipetting technique. Subsequent results were within ± 2 SDI.
3.38	10A-20B (Number B)	Partec instruments®	Seven of the twelve trials results were very high or extremely low. Had training on first submission. Thereafter, no support.	Corrective solutions offered. Suggested basic maintenance and repair training as well as technical training.
	17A-18B (Number C)	Partec instruments®	Non submission of results for two trials.	No support to service instrument. Waited for spare parts.
	20A-B (Number C)	Partec instruments®	High absolute CD4 counts submitted.	Corrective solutions offered. Suggested technical training. Subsequently changed technology to SP PLG (Trial 21) with successful performance.
	Prior 18 (Number D)	Partec instruments®	Asked advice before installation of instrument	All subsequent results were within ± 2 SDI.

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