



Report of the Workshop

QA/QC of CD4 and Viral Load Assays in the Resource-Limited Setting

Forum for Collaborative HIV Research

Department of Health Policy

School of Public Health and Health Services

The George Washington University

October 30, 2003

Warsaw, Poland

Acknowledgements

This report summarizes the discussion at the “QA/QC of CD4 and Viral Load Assays in the Resource-Limited Setting” workshop held on October 20, 2003 in Warsaw, Poland. We are grateful to Alan Landay, chair for this workshop for his continued leadership in this project.

The Forum would also like to thank the Bill and Melinda Gates Foundation, the Doris Duke Charitable Foundation and the Centers for Disease Control and Prevention for supporting this project.

A special thank you goes to Ben Collins and Houtan Mova. Without their expert coordination and support, this workshop would not have become a reality.

Finally, we would like to thank Kevin Moody in preparing this report.

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Executive Summary

The Global Fund for AIDS, Malaria and Tuberculosis (GFATM) has distributed millions of dollars globally to address HIV/AIDS in resource-limited countries. The money has gone towards prevention and treatment programs with the aim to initiate or to scale up treatment in the recipient countries. In addition, other funding agencies and international non-governmental organizations (NGOs) have been involved with smaller-scale treatment projects in the past few years. The World Health Organization's (WHO) commitment to scale up antiretroviral treatment for 3 million people by 2005 is expected to result in a surge of new, large-scale treatment projects. Furthermore, the goal of President Bush's Emergency Plan for AIDS Relief (PEPFAR) is to provide antiretroviral treatment to two million people over the next five years.

Unfortunately, most treatment programs do not address adequately enough the issue of monitoring CD4 cell counts and viral load. The WHO's HIV treatment guidelines for resource-limited settings simplifies the requirements for treatment monitoring, relying heavily on clinical symptoms. However, it is important that patients in developing countries receive adequate monitoring to ensure good treatment outcomes, prevent drug resistance and manage side effects. Cost, as well as assay complexity and equipment requirements render the state-of-the-art recommended monitoring technologies used in the developed world not suitable for most resource-limited settings. Simpler alternative technologies may be or may become available, but the integration of these into treatment settings will require a commitment from all stakeholders involved in treatment programs to ensure satisfactory clinical validation, training of laboratory personnel and maintenance of quality assurance and quality control (QA/QC).

In April 2002, the Forum for Collaborative HIV Research, an independent organization representing all stakeholders in HIV/AIDS research and funded by government and industry, held a meeting to discuss alternative CD4 and viral load technologies. During the 2002 workshop, summarized in the report, *Transfer of HIV Diagnostic and Monitoring Technologies to the Resource-Poor Setting* (available at www.hivforum.org), various assays suitable for use in resource-limited settings were identified; a process for clinical validation of these assays was outlined and collaborative working groups to continue

working on the issues were established. Based on recommendations of the 2002 workshop, the Forum convened a second workshop: *Quality Assurance and Quality Control (QA/QC) of CD4 and Viral Load Assays in the Resource-Limited Settings* on October 30, 2003 in Warsaw, Poland, the proceedings of which are described in this report. The goals for this workshop were:

- To identify what QA/QC of CD4 and viral load assays is being conducted in the resource-limited settings
- To identify the role of each of the groups in the QA/QC process
- To identify what is needed to ensure that QA/QC can be performed on all CD4 and viral load assays

Needs in resource-limited countries are diverse. Several countries have state-of-the-art CD4 and viral load monitoring technologies available at the capital city level. Others have monitoring capabilities at regional and even local level. There are, however, many countries or regions without any facilities for CD4 or viral load monitoring at all. Furthermore, QA/QC programs are minimal or non-existent in most settings. Various government institutions in North America and Europe have set up programs including QA/QC components for developing countries. Following are some examples of these:

- The French ANRS (Agence Nationale de Recherches sur le SIDA) has developed programs to facilitate cooperation and partnership with the local scientists and physicians. The ANRS is studying alternative tests for viral load and has implemented Real Time PCR in six sites in Africa and Asia. QA/QC related programs include a laboratory network for viral load assays and QA/QC protocols for clinical research as well as treatment settings. Future plans include an investigation of the impact of HIV diversity (sub-types) on assay performance.
- The US Centers for Disease Control and Prevention's (CDC) program provides laboratory support for the Global AIDS Program (GAP) in Africa, Asia and South America, including training, capacity-building, technology transfer and support for laboratory QA/QC. The latter includes provision of technical expertise for implementation and maintenance of quality, external quality assessment programs and training of laboratory personnel in QA/QC programs. CDC is

collaborating with the World Health Organization (WHO), Health Canada, and in-country laboratories to test a QA/QC procedure for manual CD4 assays. The CDC is also assisting Brazil in developing non-infectious HIV for use in its QA/QC program for viral load testing.

- The WHO views quality laboratory services along four main lines: appropriate technology, affordable prices, capacity building and reliable laboratory systems. These are addressed through the evaluation of assays and voluntary counselling and testing strategies; bulk procurement schemes; guideline development and training programs and implementation of quality management and the monitoring of the quality of laboratory performance.
- Health Canada's External Quality Assessment Program (EQAP) provides a service to monitor the QA of CD4 assays in developing countries, including selection of most suitable quality assessment materials, the delivery of rapid performance assessment, the development of a multi-level distribution network and the provision of enhanced skills building activities. The Canadian government is committed to provide support for the QA of CD4 assays to all countries currently not served by other programs. Future plans include projects with emerging technologies and reagents; better external QC systems; cost-effective shipping and multilingual materials.
- The United Kingdom National External Quality Assessment Service (UK NEQAS) conducts a monitoring program for T cell assays performed at national sites, as well as in developing countries. The program involves periodic QA testing of assay results using a stabilized whole blood product (Transfix). A recently established cost-free website with user-driven data entry is expected to enhance the quality of analysis and reports.
- The National Institute of Allergy and Infectious Diseases' (NIAID) Virology Quality Assessment (VQA) program addresses QA/QC as well as quality assessment and assay development in the United States and in some resource-limited countries. The VQA serves approximately 70 sites globally and its proficiency programs include HIV cocultures, qualitative HIV DNA, quantitative HIV DNA,

genotypic HIV resistance and HIV p24 antigen enzyme immunoassay (EIA). Challenges in developing countries include communications (English proficiency and in-country hierarchies), variable levels of expertise in laboratories (especially for QA/QC) and access to kits and disposables.

- The NIAID's Immunology Quality Assessment (IQA) Program helps immunologists evaluate and enhance the integrity and comparability of laboratory determinations performed on samples from patients enrolled in multi-center HIV/AIDS clinical trials. International projects include the CD4 international validation study, evaluation of whole blood stabilizers, validation of simpler CD4 cell subsets, designing laboratories for resource-limited countries, training personnel, recommending equipment purchases and validating laboratories.

The private sector also plays a significant role in the implementation and maintenance of QA/QC programs. The development and manufacture of equipment appropriate to the needs of resource-limited countries will be crucial as treatment programs increase in number and size. A list of technologies currently available or in development is described in Appendices B and C. Clearly, industry responsibilities include assurance of instrument and reagent quality and provision of supportive service and maintenance programs. For example, Becton Dickinson has numerous FACScout machines on the ground and has demonstrated the feasibility of obtaining good quality results on resource-limited settings. Beckman Coulter's reagents have extended stability of open-vial and closed-vial reagents. Provision of calibration and controls is another function of industry. The private sector's role needs to be expanded to collaboration and partnership with agencies and NGOs. Examples of this are the Dynal collaboration with the WHO in the search for suitable reagents for use with light microscopes and Cavid's collaboration with the NIH to document assay performance and reproducibility. Industry has a role in supporting comparative trials with gold-standard technologies and with state-of-art "alternative" technologies once these have been identified. These include performance studies, as Guava has recently completed, as well as trials for clinical validation. Training of laboratory personnel not only in assay performance but also in Good Laboratory Practice and QA/QC procedures is another opportunity for industry to partner with academia, government agencies and NGOs.

The meeting participants felt that for resource-limited settings, development efforts should focus on ease of use and tolerance of adverse conditions (e.g. high ambient temperatures, humidity, etc) rather than on the ability to test more than just a few key parameters. Of paramount importance is cost reduction, including assay price as well as maintenance, reagents and accessories cost.

QA/QC of CD4 and viral load assays must become a critical component of antiretroviral treatment programs. Donors, including the GFATM, must recognize the need for QA/QC and fund programs that incorporate monitoring mechanisms. The WHO must incorporate QA/QC monitoring of assays into its 3 million by 2005 program.

There needs to be a better mechanism for exchanging information on programs. There is considerable overlap of effort and information exchange will help promote synergies and efficiencies. Commercial availability of products such as the UK NEQAS "Transfix" product will contribute significantly to performance improvement in resource-limited setting laboratories. There is also an urgent need to work together with clinicians to determine sensitivity requirements of each assay.

What is needed on the Ground?

The potential for access to antiretroviral drugs has increased substantially in resource-limited settings due in large part to significant price reductions (to as low as US\$0.36 per day) via a variety of mechanisms. Thus drug availability is no longer the pressing issue it once was. This change has not been accompanied by parallel improvements in access to supportive diagnostic testing and laboratory monitoring of people on antiretroviral therapies. The WHO HIV/AIDS treatment guidelines (http://www.who.int/3by5/publications/documents/arv_guidelines/en/) recommendations are to base treatment initiation decisions as well as monitoring of treatment effectiveness on clinical symptoms in regions where no laboratory tests are available, or on minimal CD4 cell count testing where this is available. This compromise is justified based on the urgency of the antiretroviral treatment need -- no treatment programs should be postponed until more treatment monitoring is available -- but unquestionably inferior to the management of HIV/AIDS patients in the developed world. Laboratory based monitoring needs to be incorporated into treatment programs as rapidly as possible in order to better assess treatment effectiveness, help prevent drug resistance and help manage drug toxicities. The WHO's aspirations to treat 3 million people by 2005 places the urgency of this need into context.

Laboratory based monitoring programs need to be incorporated into treatment programs that are currently being set up or planned for the near future, including those funded by the Global Fund to Fight AIDS TB and Malaria, the Emergency AIDS Relief Plan and the World Bank's Multi-Country HIV/AIDS Program (MAP). There needs to be a large capacity building effort in order to ensure that laboratories in the resource-limited setting are set up to perform these assays, as well as the training of laboratory technicians and clinicians so that these assays can be appropriately utilized. Additionally, a bulk procurement and distribution mechanism needs to be in place, as well as a mechanism for the QA/QC of the assays.

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Technologies to the Resource-Poor Setting (available at www.hivforum.org), various assays suitable for use in resource-limited settings were identified; a process for clinical validation of these assays was outlined and collaborative working groups to continue working on the issues were established. Based on recommendations of the 2002 workshop, the Forum convened a second workshop: *Quality Assurance and Quality Control (QA/QC) of CD4 and Viral Load Assays in the Resource-Limited Settings* on October 30, 2003 in Warsaw, Poland, the proceedings of which are described in this report. The goals for this workshop were:

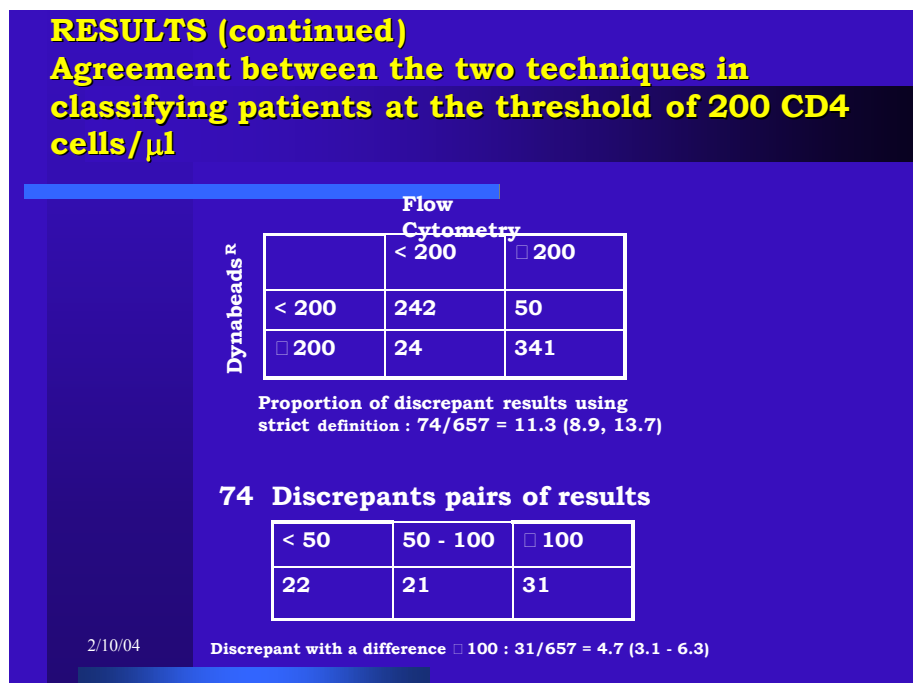
- To identify what QA/QC of CD4 and viral load assays is being conducted in the resource-limited settings
- To identify the role of each of the groups in the QA/QC process
- To identify what is needed to ensure that QA/QC can be performed on all CD4 and viral load assays

There are many efforts underway in the QA/QC of monitoring assays from both the public and private sectors. There is a need for better integrations, collaboration and networking between the different groups, so that there is a better understanding of what is available and what is being done in specific regions. A few individual perspectives illustrated below.

The Situation in Burkina Faso

In Burkina Faso, there are many initiatives working to improve access to antiretrovirals (ARVs), including those from the French Foreign Office (ESTHER), the World Bank (MAP-II and TAP), the Global Fund for AIDS, Tuberculosis and Malaria (GFATM), non-governmental organisations (i.e. MSF) and private initiatives. As a result, there are several methods in place to measure CD4 count, including FACSCan, FACSCount, Cy-Flow, CD4 Manual Count (Cytosphere) and Dynal T4 Quant (Dynabeads). Similarly, several viral load assays have been implemented including Amplicor, bDNA, Real-Time PCR and the p24 antigen assay. A network of laboratories is needed to ensure the quality of CD4 count and viral load measurement methods.

In Burkina Faso, studies have been conducted to compare Dynabeads with flow cytometry in the measurement of CD4 counts. In 657 samples from 301 patients, the two techniques differed 11.3% of the time, when using the threshold of 200 cells/ μ L. Among the 74 discrepant pairs of values, only 31 (4.7%) exhibited a difference of greater than 100 cells/uL. ¹



In general, the coefficient of variation between the reference site and remote sites was smaller with Dynabeads than with flow cytometry, showing greater reliability of the results for Dynabeads. Longer delay in handling resulted in a greater likelihood of decreased CD4 cell counts for the Dynabead assay.

A pilot study has been initiated to identify, evaluate, validate and compare available tools for quality assurance/quality control (QA/QC) of CD4 measurements using the Dynal T4 Count (Dynabeads) and Beckman Coulter CD4 Manual Count (Cytosphere); validate in the field the usefulness of a better tool in a network of national laboratories; and build a network via the Internet of national laboratories routinely using these techniques.

¹ Diagbouga S, et al. Successful implementation of a low-cost method for enumerating CD4+ T lymphocytes in resource-limited settings: the ANRS 12-26 study. *AIDS* 2003;17:2201-2208

Quality assurance procedures used include handbooks of laboratory safety; consensus conferences and international guidelines (UK NEQAS, NIH, IQA, QASI, NCCLS, CQAP, CAP); checking quality of blood specimens; avoiding microbial contamination of reagents; using appropriate anticoagulant for blood collection; homogenising reagents in solution before using; data storage; and processing of blood specimens.

Quality control procedures include internal quality control; external quality control; inter-laboratory variability; inter-technician variability; intra-laboratory variability; delay in sample handling; and reproducibility.

Tools that can be used for quality control purposes for the evaluation of CD4 assays using human stabilised cells include Ortho Absolute Control (Ortho Diagnostics Systems, Raritan, NJ); StatusFlow mid and low (R & D Systems, Minneapolis, MN); FluoroTrol-CD4 tri level low, mid, and normal (BioErgonomics, MN); CD-Chex Plus Low and normal CD4 (Strek Laboratories, Omaha, NE); Immuno-Trol Cells (Beckman Coulter, Miami, FL) and Coulter Cyto-Trol – Control Cells kit. For the evaluation of CD4 assays using fresh blood additioned with fixatives, Cyto-check or Transfix (UK NEQAS) can be used. Transfix can be a useful quality control tool in many ways. It can be used in several dilutions (1/5, 1/10), at several temperatures (4°C, 25°C, 37°C 45°C) and used *in situ* in the lab and after transportation of blood specimens, transportation etc.

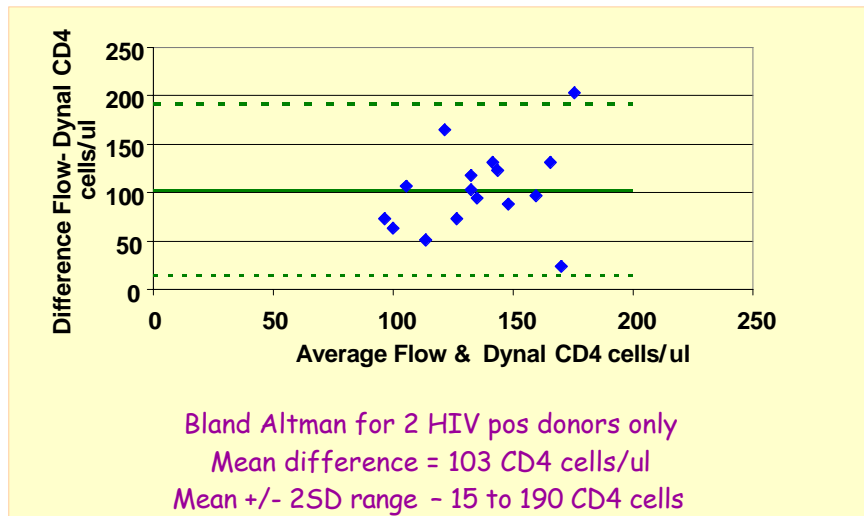
The Situation in Indonesia

The Burnet Institute in Australia has conducted programs in Bali to establish a reference facility for eastern Indonesia; form partnerships with a number of NGOs, clinicians working in HIV care, AUSAid and activists; and organize participation in an international QA program. The initial training of laboratory personnel in the use of the Dynabeads methodology took place in September 2002, followed by a series of studies (QA-1 – QA-4).

In the first study (QA-1), CD4 cell counts obtained by Dynabeads were lower than those obtained with flow cytometry and the inter-assay variation increased with increasing CD4 count. In other words, the results are more comparable at lower CD4 cell counts. The

replicate values for Dynabeads demonstrated relatively good concordance at lower levels; higher intra-assay variability was observed at higher CD4 counts.

Bali QA-1: June 2002 - Bland-Altman

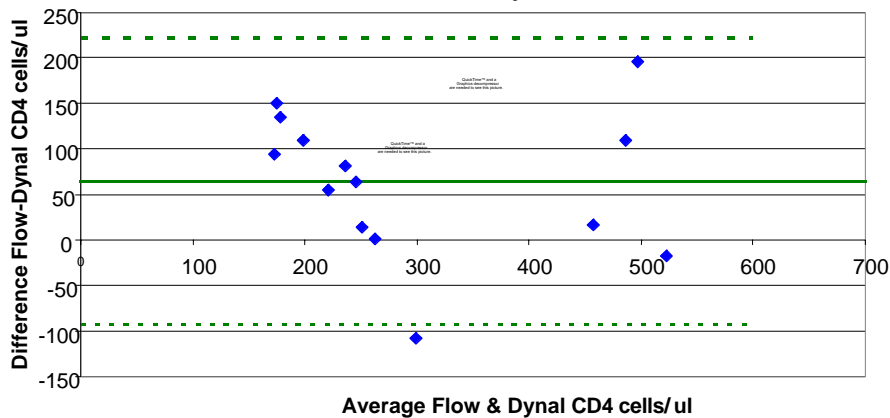


The QA-2 study performed in October 2002 was overly ambitious perhaps accounting for some of the difficulties that were encountered. The results could not be analyzed formally; nevertheless it was evident that the Dynabeads counts were much lower than those obtained through flow cytometry.

A third, much smaller study (QA-3) was undertaken, including two samples only. The results among day 5 samples comparing Dynabeads with flow cytometry were similar. Results among technicians showed good concordance.

In the QA-4 study, samples were prepared in New Jersey, Transfix was added on day 0 and then the samples were sent to Bali. On day 4, samples were analysed by Dynabeads in Bali and flow cytometry in New Jersey. The Bali results were lower by a mean of 64 cells compared to the New Jersey flow cytometry results.

Bali QA-4: April 2003



Bland Altman

Mean difference = 64 CD4 cells/ul

Mean +/- 2SD range - -93 to 221 CD4 cells

Much still needs to be done in Indonesia. The country must develop and implement a national policy on HIV/AIDS that includes the introduction of drugs and a policy for treatment monitoring. There needs to be a rational examination of the requirement for manual versus flow cytometry assays as well as for viral load assays. The Government, NGOs, clinicians and the community must develop a better relationship in-country with the companies that, in turn, should respond with more competitive pricing for kits. Finally, there must be a commitment for sustainable funding for ongoing quality assurance in order for it to be included in HIV/AIDS treatment programs.

The Situation in India

The Burnet Institute is also involved in programs in India, specifically in the cities of Chennai and Mumbai, that include the implementation of CD4 and viral load assays after a training period of one and two weeks respectively.

In a study including 123 samples conducted in Chennai, CD4 counts obtained by Cytosphere were compared to those obtained by FACSCount; the correlation coefficient (R-value) was 0.91. When grouped into categories of CD4 count (high, medium and

low), there was a very good association between the values obtained by the two different methods in all CD4 categories ($p < 0.0001$). In Mumbai, there was more variation in the results; however, the correlation between the two methods was significant for CD4 cells above 200. There were differences in the performance at the two sites and, in general, the CD4 values obtained by flow cytometry were lower than those for Cytosphere.

Programs for alternative viral load QA have not been implemented yet. Viral load QA implementation may be easier than CD4 QA because it may be possible to use the same samples that are used for RT-PCR QA. A laboratory evaluation of the Cavidix ExaVir assay showed good specificity but the replicate sample testing showed a coefficient of variance that was a little higher than obtained through bDNA.

In general, what needs to be done is the following:

- Data collection on performance of both Cytochex and Transfix with bead-based manual assays (and possibly re-evaluation for flow assays and haematol analyzers);
- Mechanism for providing kits for training programs without relying on the generosity of the companies;
- Source of funding to get training programs off the ground and to get these new labs involved long-term in QA programs;
- Further data collection on sensitivity of p24 and the ExaVir assays and an evaluation of the conversion factor for the ExaVir assay supplied by the company, with a demonstration that this is based on adequate data.

Outstanding questions include:

- Who should pay for the QA?
- Should there be a centralized viral load QA service for all resource-constrained countries participating in transfer of low cost tests?

Recommendation: Needs in Resource-Limited Settings

For CD4 assays, the focus should be on improving the performance and accuracy in the lower CD4 cell range; this is more relevant than higher CD4 cell counts for treatment initiation and treatment change decisions.

There is an urgent need to commercialize the Transfix product. Transfix is a fixative that can be added to whole blood, which allows CD4 T cell counts to remain stable for at least ten days, thus allowing for transportation of whole blood samples from the resource-limited setting for flow cytometry to regional laboratories. The UK National Health Service has no history of doing this, however this is seriously being considered and may be helped with additional encouragement from groups such as the CD4 working group of the Forum for Collaborative HIV Research's Technology Project. The standardized blood products are needed for QA/QC programs in resource-limited settings.

The issue of funding and funding sources for QA/QC programs is one of overriding concern. There is a history of QA budgets being the first to be cut in times of budgetary restrictions. For example, in the QA budget for the state laboratories in California was decreased from US\$ 6 million to US\$ 1 million QA/QC programs will be integrated into quality treatment programs only when and if there is commitment to dedicate adequate funding and budget for these programs up front. The private sector has a stake in systematic QA programs but lacks access to appropriate information. Mechanisms to incorporate the private sector interest in this process need to be explored and effective strategies for public-private collaboration in the implementation and maintenance of QA/QC programs established.

There is a need to catalog not only the assay methods themselves but also the QA methods that go with them. Guidelines for QA programs should be developed in parallel with assay guidelines. Furthermore, simple yet crucial QA procedures, such as calibration of pipettes, should not be forgotten. It is also important to determine which procedures need to be field-tested. Information regarding all QA parameters must be updated and shared with all interested parties on a regular basis.

Another important consideration is the accuracy of the tests. Clinicians should be consulted to determine whether these tests should be accurate within a few points or whether broad categories are sufficient to make good clinical decisions. A less restrictive accuracy requirement will decrease cost, complexity and QA procedures of assays.

The needs of all levels -- central, regional and local – should be considered in the process of transferring technologies. Whether QA programs are best centralized or implemented at local levels is a question that needs to be considered. It may be adequate, depending on the setting, to centralize QA testing of regional and local laboratories until point-of-care diagnostic technologies are developed and implemented sometime in the future.

The Role of Government Agencies

The role of the ANRS

The goals of the Agence Nationale de Recherches sur le SIDA (ANRS) programs in sites in developing countries are to increase effectiveness of treatment programs, to concentrate efforts and to facilitate cooperation and partnership with the local scientists and physicians. ANRS programs in the developing world include therapeutic research, basic science, social and behavioural science and health economics research.

The ANRS is studying alternative methods for viral load and has identified six sites to implement Real-Time PCR. A network of laboratories using the same standard protocol for HIV RNA quantification has been set up and this low-cost technology for monitoring patients is included within therapeutic trials conducted onsite.

The ANRS has developed the concept of “generic tests”, which involves the bulk purchase of reagents rather than kits, which is less expensive. The generic tests and Real-Time PCR have been used in Abidjan, Cote d’Ivoire and quality control work within the network of sites using this method has been completed. Real-Time PCR uses florescence that is measured in real time. It is rapid, easy, reproducible and inexpensive on a per-test basis.

In Abidjan, samples tested included those from the Prevention of Mother To Child Transmission (PMTCT) program, recently infected adults, children and the Trivacan structured treatment interruption trial. Good reproducibility was demonstrated within the different types of patient samples. The within-run variation of clinical samples showed good reproducibility with an R value of 0.99. The coefficient of variation of the inter-run variation of a weak positive control was 4.6%.

Results obtained using bDNA were similar to those obtained using Real-Time PCR. Sensitivity and specificity of Real-Time PCR were 100%. All Real-Time PCR results for uninfected children were negative but there were some false positive results with the bDNA assay. In general, there was good correlation between Real-Time PCR and bDNA with minimal number of discordant cases.

HIV-1 pediatric diagnosis: Sensitivity and specificity of the HIV-1 RNA real-time PCR assay (ANRS 1201/1202 DITRAME-PLUS)

Z	Sensitivity		Specificity		
	Age	n/N (%)	95% CI	n/N (%)	95% CI
	Day 1-8	10/10 (100%)	69.1-100.0	nd	nd
	Week 4-6	44/44 (100%)	92.0-100.0	210/210 (100%)	98.3-100.0
	Month 3-6	9/9 (100%)	66.4-100.0	nd	nd
	TOTAL	63/63 = 100%	94.3-100.0	210/210 = 100%	98.3-100.0

nd, not determined.

Sensitivity: number of positive (>300 cp/mL) samples by real-time PCR (n) / number of positive (>5000 cp/mL) samples by bDNA in HIV-1-infected children (N).

Specificity: number of negative (<300 cp/mL) samples by real-time PCR (n) / number of negative (<250 cp/mL) samples by bDNA in uninfected children (N).

20 samples from uninfected children : positive with B DNA : all negative by PCR in real time :

- child #1208-1, D2, indeterminate bDNA (3.05 Log₁₀/mL), negative by real-time PCR
- child #1163-2, W4, indeterminate bDNA (3.41 Log₁₀/mL), negative by real-time PCR

**F Rouet, IAS, Paris 2003
(TuPe277)**

HIV-1 is characterized by a high degree of diversity; nine major subtypes have been identified, plus a significant and growing number of circulating recombinant forms (CRFs) and mosaic species. This is especially true for regions with multiple co-existing subtypes, such as Africa and parts of Asia. This diversity presents challenges for

technology development. The flexibility of Real-Time PCR with respect to primer and probe provides an advantage in dealing with this challenge. Moreover, Real-Time PCR can be adapted for multiple virus detection (e.g. HBV, HCV, etc).

The ANRS has established a QC protocol for Real-Time PCR. The protocol, including the sequence of primers and the probe located in the LTR gene, has been distributed to all laboratories. Nine coded samples were sent to all six of the sites in Africa and Asia and ten sites in France for viral quantification. The objectives of this QC exercise were to test feasibility, to evaluate the training and to identify possible specific problems at each site. All samples were tested twice by two different techniques. Based on the results of a series of one-fifth dilution wells, a theoretical regression for each lab was calculated.

The sensitivity based on the QC tests was 77% (2.4 log copies/mL) and specificity was 98%. Slopes calculated on the theoretical regression were generally good; discrepancies were concentrated in the lower viral load values. This first training exercise demonstrated that even without substantial previous experience, these laboratories were able to produce consistent and reproducible results. Heterogeneous results were observed only for the sample with a low level near the cut-off value (2.4 logs). Good intra-assay reproducibility was observed, indicating that there may not be a need for duplicate sample testing. The global performance of the test is close to those of commercial tests. In all sites, at least one technician has been trained: all of the trained technicians felt that the Real-Time PCR technique is easy and rapid to perform.

The study with the 'generic test' addressed the issue of purchasing commercial kit independent reagents, such as the "mix" for PCR. This study demonstrated that this is indeed possible in African countries, while it was more difficult to organize in Asia. The laboratory in Abidjan, even during the current very difficult period of war, was able to obtain reagents much more easily than the commercial kits with more than 200 tests being done routinely per week.

According to a cost estimate for one test performed in Abidjan, Real-Time PCR can be performed for €10 per sample, including transport and taxes. This price did not include potential additional discounts that might be obtained through negotiations. The start-up costs of the instrumentation are between €30,000 to €80,000, depending on the material

used. The first generation laser was more expensive than the diode version that is currently being purchased. The initial cost is high but the fact that these instruments can also be used for other types of tests helps to justify the investment. Currently, test costs are financed through treatment programs.

This program will continue addressing different questions associated with standardization. The first focus will be on the question of HIV-1 genetic diversity in the different regions of ANRS sites.

The role of the CDC

The Centers for Disease Control and Prevention (CDC) provide laboratory support for the Global AIDS Program (GAP). This support involves coordinating technical and programmatic lab support from CDC and partner organisations. Specifically, this includes the creation of best-practice laboratory policies, the facilitation of supply, reagent and material procurement, and the provision of network opportunities for GAP laboratory directors.

The CDC is committed to reinforce technical capacity in response to GAP country needs, including the coordination of new laboratory products and technologies, as well as providing laboratory and management expertise in the areas of HIV/AIDS, tuberculosis (TB), Sexually Transmitted Diseases (STDs) and opportunistic infections (OIs).

The CDC strengthens laboratory systems by conducting in-depth assessments and preparing implementation reports; strengthening testing capacity and capability at the national, regional, and local levels; assuring coordination and communication linkages among in-country laboratories; and integrating quality laboratory services across prevention and care programs.

The CDC implements comprehensive laboratory quality assurance programs designed to provide technical expertise to implement and maintain quality assurance programs as well as ensure best use of national and international standards of quality assurance. The programs strengthen external quality assessment programs for HIV, STDs, TB, and OI

testing. The programs train international laboratory scientists in HIV testing methodologies, QA and QC program plans, and strategies for equipment procurement through “linkage” laboratories in the US.

Laboratory training is an important component to the support that the CDC gives the GAP countries. This involves developing training materials; planning, facilitating, conducting, and evaluating laboratory training courses; developing management training to promote leadership and capabilities in problem solving; evaluating current methodologies for HIV testing, related laboratory programs and best practices; utilizing a variety of training delivery methods including traditional classroom, “course-in-a-box”, and distance-based; and hosting GAP country laboratory technicians and managers at US conferences.

There are several technical support units associated with the CDC programs. The National Center for HIV, STD, and TB Prevention/Global AIDS Program (NCHSTP/GAP) hosts the Laboratory Support Team which coordinates and facilitates the efforts of CDC laboratory scientists and other professional laboratory partners to improve laboratory capabilities for surveillance, prevention, and care activities in GAP countries. The National Center for HIV, STD, and TB Prevention/Division of AIDS, STD, and TB Laboratory Research (NCHSTP/DASTLR) provides technical expertise for evaluating and implementing laboratory methods. The Division of Laboratory Systems, Public Health Practice Program Office (PHPPO/DLS) focuses on improving the quality of laboratory practices through assessment, standards, and training. It develops effective laboratory systems by providing global leadership in laboratory practices and fostering partnerships and collaborations. The Association of Public Health Laboratories (APHL) provides access to member public health laboratories and staff who provide the technical and organizational leadership for the public health laboratory network in the US.

Specific projects that the CDC is participating in include collaboration with the World Health Organization (WHO), Health Canada, and in-country laboratories to test a QA/QC procedure for manual CD4 assays. The CDC is also assisting Brazil in developing non-infectious HIV for use in its QA/QC program for viral load testing.

The role of the WHO

The WHO has a four-point strategy for addressing laboratory services, comprising appropriate technology, affordable prices, capacity building and reliable laboratory systems. Appropriate technology involves the evaluation of diagnostics tests for HIV and the development of appropriate testing strategies for voluntary counselling and testing (VCT) services. The focus in resource-limited settings is on simplicity, high quality, appropriateness and essentiality.

Affordable prices can be facilitated through bulk procurement schemes. Tests that will be procured in 2004 include HIV, CD4, viral load, HIV DNA tests, as well as equipment and maintenance contracts. More information on the bulk procurement scheme will be available soon on the WHO website. The related document, "Sources and prices of selected drugs and diagnostics for people living with HIV/AIDS" is currently already available on the WHO website.

Capacity building is facilitated by developing guidelines, training programs, workshops, etc. The strengthening of clinical laboratory services includes screening for anemia; basic clinical laboratory tests including liver and renal function tests; management of opportunistic infections; and quality assurance and equipment maintenance, including microscopes. Reliable laboratory systems can be facilitated by the implementation of quality management and the monitoring of the quality of laboratory performance.

For 2004 to 2005 and beyond, WHO's focus will be on scaling up diagnostic support related to VCT; continuing to ensure safe blood through appropriate diagnostics; expanding the bulk procurement scheme and supplies management; reinforcing basic laboratory services, including laboratory monitoring of ARV therapy at district and centralized hospitals; and scaling up activities related to HIV/AIDS diagnostic support and laboratory issues.

The role of Health Canada

Health Canada's Quality Assessment and Standardization of Immunological measures relevant to HIV AIDS (QASI) involves the continuous selection of the most suitable

quality assessment materials, the delivery of rapid performance assessment, the development of a multi-level distribution network and the provision of enhanced skills building activities. The QASI program delivery is facilitated from Ottawa by Health Canada. It is an international program that promotes, aids and supports laboratory infrastructure to deliver effective treatment of individuals living with HIV where it is needed the most. QASI also supports implementation of self-administered regional and national immunology quality assessment programs in collaboration with WHO and other international agencies.

QASI provides an external quality assessment program (EQAP) for CD4 counts where none is available. The EQAP shipments include challenge survey material with simulated specimens. The program collects, processes and analyzes EQAP data and provides rapid return of survey results to assure maximum time for remedial action. QA material is selected based on a number of criteria. It must be non-infectious and behave like whole blood. It must withstand 37-degree Celsius temperatures for three days and have a shelf-life of at least 12 days. It must also be compatible with equipment used in the field.

Over time, the coefficient of variation in the program as a whole has decreased from approximately 50% to approximately 10%, indicating successful remedial action based on timely feedback. The longer a laboratory remains in the program, the more its error rate decreases.

QASI is a global program with over 150 sites in the Americas, Europe, Africa and Asia. There are proposed shared QASI responsibility centers in Africa, separated by language. In the Anglo-African region, WHO sponsored implementation started in early 2003, while in the Franco-African region, WHO sponsored implementation began in late 2003. There will be national EQAPs for both regions. Health Canada has made a commitment to provide QASI for all non-GAP countries in Africa.

QASI supplies stable quality assessment material, provides multi-language documentation, provides statistical analysis and performance reports, assists with the writing of national guidelines, provides skills building, assists with the transfer of EQAP

to regional/national control and has pioneered the “QASI-Lympho-Site” a web based interactive EQAP

Skills-building workshops are used to transfer the EQAP management process to local and regional coordinators when possible; train laboratory personnel on how to collect, process and analyze EQAP data; and produce and distribute global performance reports. The first workshop took place during the Vancouver AIDS Conference in 1996 and several have been held since.

Brazil has completed a study, comparing their laboratory performance to the rest of the world. Their error rate is better because they are universally using FACSCount. The program provides rudimentary statistics in the form of a report to the laboratory, including mean, standard deviation, residual and standard deviation index. Each laboratory is able to compare its results with those of other laboratories.

Future projects include investigating emerging technologies for cost effectiveness and robustness; developing better external quality control systems; developing challenge surveys for manual and new alternative methods; researching the most cost effective shipping and packaging; evaluating new commercial stabilized whole blood products and preparing multilingual training material and survey documents.

The role of UK NEQAS

The United Kingdom National External Quality Assessment Service (UK NEQAS) provides information and advice to clinical laboratories on the quality of their analytical and interpretive performance so that clinicians can provide optimal care to their patients.

The UK NEQAS immune monitoring program uses two specimens per trial of stabilized whole blood; CD3, CD3/CD4, CD3/CD8 percentage and absolute values are ascertained. The consensus mean plus or minus 2 standard deviation values are determined. Performance monitoring is done using a scoring system. The maximum number of points per sample is 6; zero points are assigned if no results returned. A rolling window of 6 specimens is analyzed for performance monitoring. Two points are assigned for a result within ± 1 SD; 1 point $>\pm 1$ but $<\pm 2$; 0 points if $>\pm 2$ SD. A score of

less than 15 points indicates unsatisfactory performance and triggers remedial actions. Results show that the performance of the laboratories is improving over time.

The recently developed database-driven website with user-driven data entry will considerably enhance the process. It allows for common numbering among all UK NEQAS programs, produces higher quality reports and statistics online and allows for the creation of specific cohorts, facilitating statistical analysis on these specific groups. The use of the web service is free and users have direct access to the database, which contains historical results. Graphs can be printed or downloaded electronically in full color. The website will automatically generate emails for follow up and will help improve the turnaround time of results. The website's storing capacity (with resulting availability of these data to online users) is substantial. An additional benefit provided by the web-based approach is the significant cost savings, which will be passed on to the users. There are 50 countries in the program currently at a cost of £350 per year and this cost is expected to decrease once the website becomes fully operational. UK NEQAS does not provide translations but the website is quite simple, making it easier for non-English speaking users.

The role of the VQA

The mission of Virology Quality Assurance (VQA) program is to address: *quality control* through the monitoring of sources of error; *quality assurance* through the standardization of assays; *quality assessment* through the development of a proficiency test; and *assay development* through formulation, development and validation of new technologies.

The VQA Program is funded by the Division of AIDS (DAIDS) within the National Institute of Allergy and Infectious Diseases (NIAID) and serves approximately 70 DAIDS-supported or collaborative research sites:

- Adult & Pediatric AIDS Clinical Trials Units (ACTUs)
- AIDS Vaccine Evaluation Units (AVEUs)
- Women and Infants Transmission Study (WITS) sites
- Multicenter AIDS Cohort Study (MACS) sites
- Community Program for Clinical Research on AIDS (CPCRA) sites
- Division of AIDS Treatment Research Initiative (DATRI) sites

- National Institute of Child Health & Development (NICHD) research sites
- HIV Preventive Trials Network (PTN)
- HIV Vaccine Trials Network (VTN)

VQA proficiency programs include the following assays: HIV cocultures, qualitative HIV DNA, quantitative HIV DNA, genotypic HIV resistance and HIV p24 antigen EIA. The VQA has clients in South America, the Caribbean, Canada, Asia, Australia, Europe and Africa.

Communications is a concern with international clients, particularly with regards to direct versus indirect communication, English translation, contact information and within-country clinical trial group interactions. Import permits, customs and other governmental controls can be problematic. The level of expertise within laboratories, especially with respect to assay procedures, regulations and laboratory QA/QC can be variable.

Other concerns include access to kits and disposables. Some laboratories even wash out and reuse pipettes. The type of instrumentation and the level of service for the assays can be difficult. The degree of computerization is important, as is whether or not data is entered manually. The use of screens designed for inputting data does not necessarily eliminate problems and error rates may be as high as 20%. Logistics and timeliness can also be problematic. For example, some laboratories respond to emails within 24 hours whereas others do not communicate for months at a time.

The role of the IQA

The mission of the DAIDS funded Immunology Quality Assessment Program (IQA) is to help immunologists evaluate and enhance the integrity and comparability of laboratory determinations performed on samples from patients enrolled in multi-center HIV/AIDS clinical trials.

Several assays are evaluated including alternative measurements of CD4 (i.e. FACSCount, Guava, Dynabeads, Cytospheres), lymphocyte proliferative assays (LPA), ELISPOT and cryopreservation.

International projects at the IQA include the CD4 international validation study, evaluation of whole blood stabilizers, validation of simpler CD4 cell subsets, designing laboratories for resource-poor countries, training personnel, suggesting equipment purchases and validating laboratories.

One of the problems in international programs is the exodus of well-trained laboratory personnel to countries that pay better. The IQA is trying to address this by offering better pay than what is paid in the rest of the country. This offer may, indeed, lead to a drain of technically competent laboratory workers from the public health system and this is something that will need to be addressed.

Currently IQA focuses solely on HIV/AIDS but the program will expand to other diseases with the ultimate goal of building capacity within the public health system.

Role of Industry

Becton Dickinson

Becton Dickinson has a team dedicated to HIV/AIDS. A significant number of FACSCount instruments have been installed in developing countries; experience has demonstrated that good quality can be maintained in extreme conditions. Becton Dickinson's strong technical support program complemented by a continuous scientific support program has contributed to quality performance on the ground. The FACSCount is manufactured under good manufacturing practice (GMP) guidelines and is approved by the US Food and Drug Administration (FDA). Values obtained by FACSCount are correlated with FACSCan and FACSCalibur.

Equipment is used in a variety of settings worldwide by a variety of different NGOs. Data collection is done at the central level (FACSCalibur) and peripheral level (FACSCount).

Future plans include an optimized reagent program, allowing for a cost reduction, based on a reference test (gold standard system, reagents and data). The implementation of this new program will require validation and correlation data. There are also plans for an

optimized instrument program, allowing for a centralized reference site and peripheral screening and monitoring sites.

It will be possible to correlate CD4 cell counts obtained from other assays to those obtained by FACScan/FACSCaliber. This may be clinically useful in situations in which complete accuracy is not required for clinical decision making. Ultimately, there should not be differences in quality standards applied to the developing world compared to those required by the developed world.

Workshop participants raised the issue of equipment cost associated with this program. The instrument and reagent costs are still very high although the technology has been available for a considerable time. Furthermore, the cost of maintenance and service increases the overall operating costs even more. Industry participants responded that their options were restricted because they are answerable to their shareholders. These discussions led to the recommendation for the Forum workshop group to identify minimum quality and performance standards that the industry must meet, as this may answer contrary demands from its shareholders.

Beckman Coulter

Beckman Coulter produces several CD4 cell count assays, including dual platform, single platform and CD4 manual count assays. The company also produces many reagents including panleukogating reagents, 2-colour IVD reagents, triChrome IVD reagents, tetraChrome and tetraOne reagents. The ImmoPrep reagent systems (IVD) have good stability at cold temperatures and can handle specimens up to 72 hours old. Beckman Coulter also offers several calibrators and controls for flow cytometry-based CD4 assays.

The relevance to resource-limited settings is that the products have significant “open-vial” stability for some reagents; there is extended “closed vial” stability of some reagents at temperatures of 25 and 37 deg C; and there is extended stability of samples prepared using ImmunoPrep reagents. There is the possibility of using some QA/QC reagents across platforms.

There was a feeling among the group that there is a need to simplify the tests. Is there really a need to do CD3 and CD8 counts? Would it not be possible to simplify and reduce the cost to patients and programs if these were excluded? The industry participants indicated that machines are only produced for markets that can afford them. Where markets do not exist, machines will not be produced.

Partec

The Partec flow cytometer, CyFlow, is a flexible platform capable of measuring 1 to 5 parameters and is designed for low-resource settings. It is a volumetric instrument that triggers on color instead of on scatter. It uses generic monoclonal antibodies a no-lyse, no-wash procedure, and the cost is approximately two dollars per test. CyFlow uses laser technology that doesn't require cooling. The machine costs about US \$21,000.

A study conducted by the California Department of Health Services was performed to compare CyFlow with FACSCalibur: There was good correlation for CD4 counts ($R=0.975$) but CyFlow results were approximately 10.5% lower. There was also good correlation for CD8 counts ($R=0.983$), with CyFlow results approximately 12.3% lower.

Partec had previously reported that the values obtained with CyFlow should be higher than FACSCalibur, not lower, because of the no-lyse procedure. The discrepancy between the prediction and actual data may be due to a volumetric problem. One participant suggested that the discrepancy may also be due to viscosity changes and this needs to be investigated. There was general agreement that this assay needs to be externally validated.

Dynal Biotech

Dynal Biotech is developing the Dynal T4 Quant kit in France. Numerous groups around the world are evaluating this assay.

There have been numerous publications comparing Dynabeads with flow cytometry and many NGOs and government laboratories have decided to use this technology. There is a good correlation between the two assays when CD4 counts are less than 500 cells/ul.

The WHO has done a study with centre Muraz in Burkina Faso to determine which stains are suitable for light microscopy. The manual technique restricts the number of tests that can be performed daily and it is cumbersome to count the stains with a light microscope. Currently, Dynal Biotech is working with cell counter companies to determine if it is possible to use the Dynabeads method on a cell counter directly. To QC the assay, Dynal Biotech advises that a test be done once weekly. One blood sample should be divided into three parts and given blinded within a series of tests to the technician to see if the same results are achieved from the three samples. Additionally, Dynal Biotech advises to compare the results from samples using the Dynal T4 Quant Kit to that with a flow cytometer at a reference center each month. The company is currently working with Health Canada and the Burnet Institute to determine which fixative product works best so that samples can be sent via mail.

Guava

The Guava EasyCD4 assay is a cap-loading flow cytometer with one moving part that takes in the specimen and uses a green laser. Because it is cap-loading, there is no sheath fluid and no waste. This is a no-wash test yielding absolute counts. It can be performed using lysed or no-lyse protocols. The assay uses 10µL of whole blood. CD3/4 and CD4/8 assays are available. Over 100 samples have been run and compared to regular flow cytometry. There was very good correlation, with $R^2 = 0.97$. Many replicates of the specimens have been tested by different operators and the same operators with excellent coefficients of variance. Although the marketing price of the machine is still not known, it may be possible to develop a machine with some technical modifications for about half the cost of existing machines. The cost per test is expected to be low because it uses very little blood and, therefore, very little antibody. Guava is aiming for US \$1 per test, while maintaining excellent quality.

Cavidi

Cavidi has developed a viral load quantifying method-based measurement of reverse transcriptase (ExaVir), using a gel that traps the virus. For QC/QA, Cavidi does not have an external system but rather uses an internal system. The NIH is studying this

methodology in several sites and has reported good reproducibility. The current cut-off for the ExaVir assay is about 5000 copies/mL. Cavid plans to announce an improved version of the assay with a cut-off of 1000 copies/mL. The assay time will be 3 hours longer, the colorimetric will get shorter, more lysate will be added to the well and there will be increased polymerization. It will be possible to provide a negative control for the test but a positive control will be more difficult.

Discussion, Conclusions and Recommendations

Information Exchange and Collaboration

The need for improved exchange of knowledge, experience and information cannot be overstated. Keeping all stakeholders (players in this field, interested parties) updated on progress in the area of laboratory based monitoring of HIV treatments and the related QA/QC issues around the world will help to improve collaboration, reduce redundancy and identify gaps in the coverage of QA/QC services. Workshop participants recommended several steps that would contribute to this process. These include:

- Compile a list of all international laboratories involved in QA/QC programs and publish this on one organization's website for ready access to everyone
- Include QA/QC information to the CD4 and viral load assay summary tables (Appendices B and C).
- Collaborate by sharing panels of samples with other workshop participants for cross-testing and communicate the results for speedier improvements

Ultimately however, support for infrastructure to maintain an ongoing exchange is needed.

Standards for Clinical Decision Making

A careful consideration of the sensitivity requirements for viral load and CD4 monitoring tests is crucial at this time and needs to be conducted by clinical advisory bodies. The drive to refine technology as much as scientifically possible may not be the best clinical option. In the case of CD4 and viral load assays for resource-limited settings, there is a trade-off between highly sensitive tests and tests that are affordable. These discussions need to be conducted with reference to both developed and developing world.

For CD4, it is clear the accuracy of the tests needs to be focused on the lower CD4 cell count range. The question is how sensitive the tests need to be at higher values or those reflecting the normal range. For viral load, the current cut-off is 50 copies/mL. For monitoring purposes, how precise does the viral load measurement need to be? Does it need to identify an accurate specific value or would it be sufficient to produce a test that provides results within a certain range? How frequently do patients need to be monitored? Consensus is needed from the clinicians to determine standards for diagnosis, monitoring of treatment, monitoring of side effects and the management of opportunistic infections.

A Better Instrument

Machines need to become more suited to the resource-limited setting. There is no need for a machine to do a panel of 45 different tests on one sample. In some cases, even CD8 may not be required. Simplicity of operation is very important, as is readout. The instruments should be robust and be able to resist the elements. Producers should remember that ideal lab conditions do not always exist and the machines designs need to reflect conditions in these settings.

Low Price

The market for low-cost, high-quality machines is growing fast. The WHO has committed to expanding treatment to 3 million patients by 2005 and other initiatives are already beginning to scale up, all of which translates into a growing market for low-cost HIV-related diagnostics. In general, purchasing of equipment has not been a problem for programs implementing HIV treatment programs. A much more significant problem has been the affordability of maintenance service agreements, reagents and other equipment costs. Mechanisms to decrease equipment and peripheral costs need to be found. For example, the governments of countries where treatment programs take place need to remove all taxes and tariffs related to medicines, machines, reagents and equipment. In some countries the in-country mark-up can be 40% or more of the manufacturer's price.

Paying for Monitoring and QA/QC

Donors must realise that providing antiretrovirals is not enough. Funds must be made available to monitor therapy and to check the quality of that monitoring (i.e. QA/QC). Without appropriate monitoring and checks and balances for the system, effectiveness of treatment regimen cannot be ascertained. While the absence of these monitoring tools should not prevent treatment programs from beginning, a comprehensive implementation plan for the monitoring of CD4 and viral load must be integrated into the treatment program in the early stages.. What is the best model to use for a given setting? Should one first start with centralized monitoring or with regional monitoring centers? Studies should be carried out to determine the most effective and efficient solutions so that resources dedicated to monitoring are not wasted.

Other Outstanding Issues

Transfix

The Forum for Collaborative HIV Research (FCHR) will write a letter to the UK NHS to advocate for the commercialization of Transfix and to ask for the large-scale production of 200-count standardised whole blood product.

WHO & US State Department and other Major Donors

The FCHR will impress upon major donors the need to include monitoring (and QA/QC) in treatment programs. Those submitting project proposals to donor agencies should be requested to include an implementation plan for monitoring treatment and for the implementation of QA/QC. Donor institutions should provide funding for existing laboratories to participate in international QA/QC programs.

Next Steps

The FCHR will establish a committee comprising participants of the workshop to address the actions points and next steps in the process of increasing access to quality monitoring of antiretroviral treatment in resource-limited settings.

Appendix A – Workshop Participants

The Forum for Collaborative HIV Research

QA/QC of CD4 and Viral Load Assays in the Resource-Limited Setting

Warsaw, Poland

October 30, 2003

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Appendix B – Table of CD4 Assays

CD4 Commercially Available Assays Summary

System	Key Features	Parameters measured	Advantages	Disadvantages	Materials needed	Cost of Instrument	Cost/test (excludes costs of service, labor, logistics and results delivery)
Lymphocyte Count	<ul style="list-style-type: none"> • Standard hematology analyzer • Hemacytometer count 	<ul style="list-style-type: none"> • WBC count and diff • Lymph count and percent 	<ul style="list-style-type: none"> • Already standard laboratory instrumentation and material on low end (3 part diff analysers) and high end (5 part diff analysers) • Low cost • Fully automated • Low expertise required • WHO endorsed for monitoring HIV disease stage along with clinical symptomology 	<ul style="list-style-type: none"> • Correlation to clinical assessment of drug efficacy unknown • Needs good clinical assessment with ALC for meaningful interpretation • Needs relatively fresh sample for analysis to ensure relative accuracy of WBC diff and hence ALC • Combination of ALC with PLG CD4 as a public health approach and maximum cost effectiveness of limited resources i.e. use ALC when HIV+ ambulant and well (ands patient likely to have a CD4 > than 400/ul. Reserve CD4 count for when AIDS+ and sick, and accurate CD4 crucial for ensuring patient receives correct treatment i.e. 250 cells/ul and less or ALC < 200 cells/ul 			<USD 1
Beckman Coulter Cytosphere kit	<ul style="list-style-type: none"> • Latex bead-based kit for light microscopic counting 	<ul style="list-style-type: none"> • CD4 absolute count 	<ul style="list-style-type: none"> • Simple • No instrument needed other than microscope with 40x objective and 	<ul style="list-style-type: none"> • No CD4 percentage capability, important for monitoring infants and children • Low throughput (although 	Cytosphere reagent kit containing: <ul style="list-style-type: none"> • CD4 latex beads • CD14 latex 	USD 2,000	USD 8 (cost varies by region)

CD4 Commercially Available Assays Summary

			<p>counting chamber (0.1 mm deep)</p> <ul style="list-style-type: none"> • Can be performed in remote sites without controlled utilities • IVD cleared by FDA • 0.1 ml whole blood/test • Can be used on blood up to 72 hours after collection (needs to be confirmed) 	<p>higher than Dynal)</p> <ul style="list-style-type: none"> • Moderate cost/test • Labor intensive 	<p>beads for monocyte blocking</p> <ul style="list-style-type: none"> • Specific stain 		
Dynal Manual Dynabeads assay	<ul style="list-style-type: none"> • Kit • Magnetic-bead based for fluorescent microscopy, but light microscopy can be used 	<ul style="list-style-type: none"> • CD4 absolute (or CD8 absolute; CD8 not needed for routine monitoring) 	<ul style="list-style-type: none"> • Simple, • no instrument needed other than microscope with 40x objective and counting chamber (0.1 mm deep) • can be performed in remote sites without controlled utilities • 125ul whole blood/test • Can be used on blood up to 72 hours after collection (needs to be confirmed) • Can be used with light or fluorescence microscopy 	<ul style="list-style-type: none"> • No CD4 percentage capability, important for monitoring infants and children • Low throughput • Labor intensive • Sample prep longer than Coulter • Specific magnet and rotating mixer needed • Moderate cost/test • Less accurate at higher CD4 counts (>500) 	<ul style="list-style-type: none"> • Dynal magnetic beads coupled to CD4 and CD8, and monocyte blocking CD14 beads • Available in kit configuration • CD45 also available • Specific stain (differs for light and fluorescence microscopy) 	USD 2,000-10,000	USD 4-5 (WHO distributes at 30% discount)
Becton Dickinson FACSCount	<ul style="list-style-type: none"> • Dedicated instrument system for CD4 and CD8 absolute counting 	<ul style="list-style-type: none"> • CD4 absolute count • CD8 absolute count • CD3 absolute count 	<ul style="list-style-type: none"> • Fully automated • No lysing reagents required • Low-level expertise required • IVD cleared by FDA 	<ul style="list-style-type: none"> • No CD4 percentage capability, important for monitoring infants and children • Dedicated platform, no menu expansion 	<p>BD unitized reagent kit</p> <ul style="list-style-type: none"> • CD3/CD4+ beads • CD3/CD8+ beads 	USD 25,000	USD 15-20 (may be less for developing countries) In South

CD4 Commercially Available Assays Summary

		<ul style="list-style-type: none"> • CD 4/8 ratio 	<ul style="list-style-type: none"> • Accuracy and precision validated • Can be used on blood up to 24 hours after collection • 100 uL whole blood/test • CD4 useable range: 50-2000 cells/uL 	<ul style="list-style-type: none"> • Unitized reagents • Long process time • High cost 	<ul style="list-style-type: none"> • Instrument Calibrants for each sample batch 		Africa ~USD 20-25. Pricing varies because instrument, service, shipping and consumable charges sometimes included in the overall price
Partec CyFlow	<ul style="list-style-type: none"> • Two color instrument with software and 	Dependent on reagents used (open architecture): <ul style="list-style-type: none"> • CD4 absolute count • CD8 absolute count • CD4/CD8 ratio 	<ul style="list-style-type: none"> • No lyse system for absolute counts • Volumetric cell counting, no bead calibrators required • Open software architecture for menu expansion • 15 minutes turn around time • lyse no-wash system can give percent 	<ul style="list-style-type: none"> • No CD4 percentage capability if no-lyse method is used • No independent validation of performance • No model distinction for various Cyflow models • CANNOT BE RECOMMENDED AT THIS TIME 	<ul style="list-style-type: none"> • Single color CD4 for CD4 count only • Add CD8 for two color CD4/CD8 absolute counting • Add Cyflow lyse for CD4 and CD8 percentages 	USD 22,000	Depends on reagent purchase
Panleucogating CD4 (BCI licensed from South Africa NHLS as 'PLG CD4')	<ul style="list-style-type: none"> • Reagent 2 color plus lyse for standard flow platforms and possibly Partec but not FACSCount 	<ul style="list-style-type: none"> • CD4 percent • CD4 absolute count • Single platform capability with bead calibrator: • WBC Count • Lymph percentage and absolute count 	<ul style="list-style-type: none"> • Provides both absolute and percentage for CD4 • CD45 may provide capability for 3 part diff – needs validation (CD45only) • CD4/45 can generate 5 part diff for same costs as the cheap CD4. 7 part - if blasts and nucleated RBC are 	<ul style="list-style-type: none"> • Requires flow cytometer instrument and hematology analyzer for dual platform application • No CD8 count or percent measurement • Need independent validation studies to confirm performance • No automated software currently available • Need independent validation for reagents 	<ul style="list-style-type: none"> • CD45/CD4 two color reagent • Lytic reagent – Q-prep • Optional – flow count beads 		USD 2-3 including Immunoprep in South Africa (R23 per test). Does not include

CD4 Commercially Available Assays Summary

		<ul style="list-style-type: none"> • 3 part diff? • 5 part diff possible using CD45/4 	<p>identified.</p> <ul style="list-style-type: none"> • If PLG CD45/4 SP option used, can generate a WCC and 5 part diff • May be used on all current flow cytometers (probably Partec) • Accuracy on old blood samples • Single platform or dual platform • Dual platform data correlated well with single platform standard measurements <ul style="list-style-type: none"> ○ Decreased costs - no bead calibrators required ○ <u>Reliable SINGLE TUBE analysis</u> (QC of count lies in QC of WCC on haem analyser) • Menu expansion capability • I.e. PLG concept can be applied to any cell enumeration including CD8, CD3, and CD19 etc. • PLG concept not limited to two color but can be extended to enumeration of at least 3 cell types if 	<ul style="list-style-type: none"> • Some training is required 			<p>instrument and other consumable charges</p> <p>PLG CD4 with Flow Count USD 3-5. PLG CD4 with Flow Count \$3-4 Cost including new BC XL ~placement, all PLG reagents, red cell lysing reagents and controls – USD 10-12</p>
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CD4 Commercially Available Assays Summary

			<p>four color FCM is used</p> <ul style="list-style-type: none"> • Low cost 2 color reagent + lyse • Flow expertise required • 25-30 minute turn around time • High throughput (up to 300-400 samples per day on a single instrument – 8 hour day) • No automated software 				
Standard Flow	<ul style="list-style-type: none"> • Standard one and two laser flow instruments + multiple reagent systems for CD4 counting 	<ul style="list-style-type: none"> • Dependent on reagent kits/methods • CD4 absolute and percentage • CD8 absolute and percentage • CD3 absolute and percentage • Lymphosum – T, B and NK cells • CD4/8 ratio 	<ul style="list-style-type: none"> • CDC guidelines used by many nations and labs for measurement requirements and QC • Single platform capability with bead calibrator products • Accuracy and precision validated • IVD approved reagents and systems • Industry standard reference methods • Multiple choices of reagents 	<ul style="list-style-type: none"> • High cost of instruments and reagent systems • High expertise required • Requires controlled utilities and environmental conditions • Need good technical support 	<ul style="list-style-type: none"> • Multiple reagent systems • Instrument set-up reagents • Bead calibrators • Compensation reagents • Lytic reagents 	USD 40,000-80,000	USD 12-40/test determined by what all is included in test ie, instrument charge, reagent charge, service charge, consumables

Appendix C – Table of Viral Load Assays

Viral Load Commercially Available Assays Summary

System	Key Features (cutoffs reflect manufacturer's claims and may not be based on the same criteria)	Analyte measured	Advantages	Disadvantages	Materials needed	Cost/test (excludes costs of service, labor, logistics and results delivery)
Roche Molecular Systems (AMPLICOR Monitor MWP manual and COBAS automated)	Cutoff 50 copies/ml (0.5 ml); 400 copies/ml (0.2 ml)	HIV RNA	<ul style="list-style-type: none"> • Equipment can be used for other diseases • Can be used for clades A, B, C, D, E, G • 0.2 to 0.5 ml plasma • High through-put 	<ul style="list-style-type: none"> • Contamination risk • Skilled technicians • Cost • Dedicated equipment and space • Need good technical support 	Consumables required specific for test system which must be considered in pricing	≥USD55 but varies by regions (e.g., USD17 in countries based on UN designation of resource limited)
Bayer Diagnostics (VERSANT bDNA 3.0)	Cutoff 75 copies/ml (1.0 ml)	HIV RNA	<ul style="list-style-type: none"> • Equipment can be used for other diseases • Can be used for clades A, B, C, D, E, G • High through-put 	<ul style="list-style-type: none"> • Contamination risk • Skilled technicians • Cost • Dedicated equipment and space • Need good technical support • Need 1.0 ml plasma 	Consumables required specific for test system which must be considered in pricing	>USD80
BioMerieux (Organon- Teknika NucliSens QT)	Cutoff 50 copies/ml (2.0 ml); 400 copies/ml (0.2 ml)	HIV RNA	<ul style="list-style-type: none"> • Equipment can be used for other diseases • Can be used for clades A, B, C, D • Can be used for all biological fluids and dried blood spots (sensitivity issues with blood spots) 	<ul style="list-style-type: none"> • Contamination risk • Skilled technicians • Cost • Dedicated equipment and space • Need good technical support • Need at least 1.0 mL plasma to achieve reported sensitivity 	Consumables required specific for test system which must be considered in pricing	≥USD80
Primagen Retina Rainbow	Cutoff 50 copies/ml (2.0 ml); 500 copies/ml (0.2 ml)	HIV RNA	<ul style="list-style-type: none"> • Compatible with dried fluid spots and plasma, serum, whole blood, mothers milk, etc 	<ul style="list-style-type: none"> • Contamination risk • Skilled technicians • Dedicated equipment and space • Need good technical support 	<ul style="list-style-type: none"> • Tubes, (filter-) tips, test-strips estimated cost: <USD6 anywhere in the world • Isolation reagents 	USD20

Viral Load Commercially Available Assays Summary

			<ul style="list-style-type: none"> Equipment can be used for other bio-markers 	<ul style="list-style-type: none"> Needs more extensive evaluation in clades 	needed: <USD8	
Perkin Elmer Ultrasensitive p24	<p>Cutoff approximately 30,000 copies/mL with current kit extraction reagent (0.05 ml)</p> <p>Cutoff variable due to non-virion associated p24 contribution</p>	p24 antigen	<ul style="list-style-type: none"> Equipment can be shared with ELISA Training available at CDC, UNC, Rush and through company May be used for pediatric diagnosis Easy training <1 day turn around time High through-put 	<ul style="list-style-type: none"> Dry heat block NEEDS MORE EVALUATION FOR USE IN CLINICAL MANAGEMENT- need to understand the clinical impact of non-virion associate p24 Needs more extensive evaluation in clades 	Uses same consumables as ELISA testing	<p>USD5 (kits only)</p> <p>USD10 (kits, heat block, computer, reader, washer included)</p>
Cavidi ExaVir	Cutoff approximately 5,000 copies/mL (1.0 ml) by either colorimetry or fluorimetry,	Reverse Transcriptase activity	<ul style="list-style-type: none"> Easy training Training available through company and at Melbourne, Australia Can be used for all clades (TBD)? Easy to perform assay Potential use for clinical management Can be used for NNRTI drug resistance monitoring Results reported as Fg RT/ml as well as RNA copies/ml equivalents 	<ul style="list-style-type: none"> 32° dedicated incubator and vacuum pump needed Performance time about 1.5 days (fluorimetric), 2.5 days (colorimetric) Needs more extensive evaluations for use in clinical management Needs more extensive evaluations in clades Requires 1 ml plasma Positive and negative control not supplied 	Consumable pricings need to be considered (less than USD1 per specimen)	USD10-20