

WHO consultation on technical and
operational recommendations
for scale-up of laboratory services
and monitoring HIV antiretroviral
therapy in resource-limited settings

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Abbreviations

| | |
|---------------|--|
| AIDS | acquired immunodeficiency syndrome |
| ALT | alanine aminotransferase |
| AMDS | AIDS Medicines and Diagnostics Services |
| ART | antiretroviral therapy |
| ARV | antiretroviral |
| CD4 | T-lymphocyte CD4+ |
| CPS | Contract Procurement Services (of WHO) |
| CRF | circulating recombinant form (of HIV) |
| CSF | cerebrum spinal fluid |
| DART | Development of Antiretroviral Therapy in Africa |
| EDM | Essential Drugs and Medicines Policy (WHO Department of) |
| EHT | Essential Health Technologies (WHO Department of) |
| ELISA | enzyme-linked immunosorbent assay |
| EQA | external quality assessment |
| FBC | full blood count |
| GFATM | Global Fund for AIDS, Tuberculosis and Malaria |
| GLP | good laboratory practice |
| Hb | haemoglobin |
| HIV | immunodeficiency virus |
| HIVDR | HIV drug resistance |
| MAb | monoclonal antibody |
| MSF | Médecins Sans Frontières |
| MSH | Management Sciences for Health |
| NGO | nongovernmental organization |
| NRL | National Serological Reference Laboratory (Australia) |
| NVP | nevirapine |
| OEM | original equipment manufacturer |
| OI | opportunistic infection |
| PCP | Pneumocystis carinii pneumonia |
| PCR | polymerase chain reaction |
| QA | quality assurance |
| QC | quality control |
| QM | quality management |
| RT | reverse transcriptase` |
| SOP | standard operating procedure |
| STARHS | serologic testing algorithm to detect recent seroconversion |
| STI | sexually transmissible infection |
| TB | tuberculosis |
| UN | United Nations |
| UNAIDS | Joint United Nations Programme on HIV/AIDS |
| UNFPA | United Nations Population Fund |
| UNICEF | United Nations Children's Fund |
| VCT | voluntary counselling and testing (= T&C: testing and counselling) |
| VL | viral load |
| WHO | World Health Organization |
| ZDV | zidovudine |

I. Introduction

A WHO consultation on technical and operational recommendations for scaling up laboratory services and monitoring HIV antiretroviral therapy (ART) in resource-limited settings was held on 13-15 December 2004 in Geneva.

Despite the progressive increase in access to HIV antiretroviral (ARV) drugs in countries with limited resources promoted by the 3 by 5 initiative, WHO recognizes that the current limitations on laboratory capacity in resource-poor settings can be an important barrier in the way of reaching the planned treatment target. WHO, as stated in its global HIV ART guidelines for resource-limited settings, will work together with the international community and countries to improve the laboratory infrastructure at local and regional levels so as to permit uniform availability of HIV and CD4 testing, wider availability of automated haemoglobin and chemistry testing, and national availability of viral load (VL) testing, while the capacity for HIV drug resistance testing (HIVDR) should be available at regional level. Given current local realities and future scenarios, this will require choosing uniform, simplified and cost-effective methodologies at the country level and ensuring supplies of reagents and the maintenance of equipment.

The aim of the consultation was to obtain clear and realistic guidelines as to which diagnostic and monitoring schedules were optimal and how they could be delivered in order to assist decision-making on treatment and facilitate the implementation of strategies and necessary actions for scaling up diagnosis and monitoring at the local, regional and global levels, with particular emphasis on resource-constrained settings. It was required that the resulting recommendations would provide useful tools for the rational implementation of scaling-up processes, taking into consideration variations between developing countries in human resources, health structures and socioeconomic contexts.

The objectives of the consultation were to:

- ▶ revise and update recommendations for laboratory monitoring of ART in line with a public health approach to treatment;
- ▶ review the current status of laboratory technologies relevant to a public health approach to treatment and scale-up issues (availability, cost, performance issues, personnel requirements, reliability, etc.), and to consider them in respect of guidelines for selection and procurement;
- ▶ review the current status of the WHO system and other systems for assessing the performance of technologies and reagents relevant to the laboratory monitoring of ART;
- ▶ identify the operational and innovative research agenda for laboratory tools to support wider access to ART in resource-limited settings.

The consultation was opened by Dr Jim Kim, Director of the HIV Department. Dr James Hakim chaired the meeting and Dr John Parry and Dr William Rodriguez served as rapporteurs.

Dr Kim emphasized the credibility gap that had become apparent between HIV prevention activities through behavioural change and the inadequate delivery of ART in resource-poor settings. Since the inception of the 3 by 5 initiative, many new alliances had provided a focus for activity. As a result there was an urgent need to scale up the public health response. A major deficit in this response concerned access to appropriate laboratory support, particularly for the measurement of CD4 cells. It was urgently necessary to scale up the delivery of ART in order to meet the 3 by 5 target, and doing so was highly dependent on simultaneous capacity-building of laboratory services. Funds were available to facilitate the rehabilitation of the laboratory infrastructure and they had to be used quickly. The consultation had been organized in order to seek expert advice on the requirements for the laboratory support needed to ensure effective health delivery systems.

The public health approach to antiretroviral therapy in resource-limited settings

WHO had adopted a public health approach to ART in resource-poor settings. The need was urgent because more than 8000 HIV-infected people died every day from a preventable and treatable disease. The 3 by 5 initiative aimed to deliver ART to 3 million eligible people by the end of 2005, and the ultimate goal was to achieve universal access in accordance with need. In June 2004 only 440 000 individuals were estimated to be receiving ART. The major challenges were to scale up prevention so as to retard the epidemic and to convert HIV infection from being a fatal condition to being a chronic manageable disease by strengthening health systems. A public health approach was seen as most appropriate because there were too few trained physicians for an individualistic approach to be adopted and

because laboratory support services were inadequate. Consequently, simplified standard care packages were needed for delivery by treatment teams with local adoption and adaptation. These packages would include: routine offers of testing and counselling; simplified treatment; opportunistic infection (OI) prophylaxis and regular patient review; first-line and second-line regimens; simplified clinical decision-making; simplified laboratory and clinical monitoring; standardized patient registers, drug cards, etc.; community engagement involving adherence and chronic management; and free care at the point of delivery.

The four key steps in the clinical management of ART were discussed, i.e. when to:

start therapy;

substitute for toxicity;

switch following failure;

stop and commence palliative care.

The most difficult decision, for which laboratory support was usually required, concerned the time of switching to second-line ART. Constraints of time and cost dictated that the support should be as simple as possible. WHO did not recommend VL tests for routine basic patient follow-up, nor resistance testing for individual patient care. However, these technologies could be employed for population monitoring.

Review of HIV/AIDS diagnostics and laboratory support activities

The activities of the WHO Department of Essential Health Technologies (EHT) relevant to the ART scale-up initiative were described. In response to the urgency of the 3 by 5 initiative, EHT had extended its HIV diagnostics work to activities needed for ART monitoring. In addition to the evaluations of HIV screening and diagnostic tests previously undertaken, WHO had started comparative assessments of CD4 measurement technologies and of possible alternative technologies to VL testing. The development of innovative technologies was also being supported. WHO continued to provide technical information and advice by publishing policies and guidelines for laboratory and diagnostic services and through advocacy and the sensitization of policy-makers. Essential requirements for HIV/AIDS diagnostic support in resource-limited countries were also being updated. Access to quality diagnostics, reagents and equipment was facilitated through the WHO Bulk

Procurement Scheme, which provided these commodities at negotiated prices to its Member States. Several additional initiatives were supporting the provision of good laboratory services. Needs for capacity-building were being met by the development of core curricula and training materials along with training-of-trainers workshops addressing the diverse needs of laboratories at the regional, national and local levels. Laboratory performance was being monitored by an array of WHO external quality assessment (EQA) schemes, including HIV serology, CD4 counts, haematology and clinical chemistry. In addition, training in systems of good quality and supporting quality assurance (QA) activities and management were being conducted at the national, regional and district levels.

Discussion

In some settings, access to ART seemed to be limited by the high costs of VL testing. It was stated that VL monitoring was not a requirement for monitoring ART, although prices for VL assays were falling. It was argued that manufacturers and suppliers of diagnostics needed information about market requirements at the country level and that this would permit price negotiations on the basis of expected volumes. However, WHO had always managed to secure low prices without being committed to volumes or any particular manufacturer. Prices also often depended on specific procurement routes: some countries created additional costs by imposing significant import duties, and local distributors could also introduce substantial mark-ups. Attempts to minimize these added costs were desirable.

When recommendations were being devised on the appropriateness of technology in different settings, consideration had to be given to the need for rapid access to spares, maintenance and technical support. The procurement of appropriate technologies was often impossible because of an inability to generate local data ensuring well-informed choices and because of a lack of communication between purchasers and users of technology. Moreover, local laboratory management often lacked the skills, knowledge and/or motivation to introduce technological innovations. It might be necessary to have a stronger representation of laboratory experts in national AIDS programmes and ministries of health and to pool regional expertise, as with the HIV laboratory network for African countries. Several comments were made concerning the problems of early diagnostic approaches for infants.

II. Scale-up experiences

a. Kenya

The HIV epidemic had a negative impact on all sectors of Kenyan society. Previous health gains had been reversed. Life expectancy at birth had fallen from 62 to 46 years. In the medical wards of government hospitals, 50–70% of bed occupancy was HIV-related. The prevalence of HIV in 2003 was estimated to be 7%, equivalent to 1.25 million Kenyans estimated to be HIV-positive, of which 190 000 were thought to need ART. The roll-out of ART was going well, fostered by clear political commitment at a high level. Strategic and operational plans were developed to support the 3 by 5 goal. A pragmatic public health approach to ARV provision was adopted, based on the development and application of national guidelines, standardization and quality control. The national programme aimed to deliver ART to 50% (95 000) of eligible people by 2005 and to 75% by 2008. In order to achieve these goals it was recognized that multisectoral involvement was required and that it would be necessary to work with various partners. Staff training was recognized as essential for successful implementation. The indicators of progress were very good: substantially increased numbers of people were seeking voluntary counselling and testing (VCT) and the ART sites planned for 2005 were already active in 2004. Nevertheless, there was still a long way to go in order to meet the need, strengthen the health delivery infrastructure adequately and secure sufficient funding.

b. Senegal

It was estimated that the prevalence of HIV in Senegal was approximately 1.5% (80 000 cases). Pilot studies on the introduction of ART began in 1998. In order to prepare for ART scale-up a programme of training for relevant health care personnel had been developed. About 450 people had already been trained. A national team had provided both theoretical and practical training, and ART regimens had been harmonized. Further staff development was undertaken through a system of mentoring. The Government was providing funding for HIV testing, CD4 measurements, OI prophylaxis and ART. The monitoring of ART encompassed clinical assessment and basic laboratory tests, including CD4 determinations. The structured education of patients on ART concerning the need for adherence to drug regimens was seen to be a key element of successful treatment. Access to VCT was being enhanced: three centres were planned for each region and it was intended to introduce antenatal testing and testing at tuberculosis (TB) and sexually transmissible infection (STI) clinics. At the time of the consultation, 2800 patients were receiving ART. It was intended that 7000 would be doing so by 2006.

c. Thailand

It was estimated that more than 1 million persons had had HIV infection, almost 600 000 of whom were still living with the virus and 61 394 of whom had AIDS. During 2004, approximately 20 000 new infections and nearly 50 000 new AIDS cases were expected. ART was first used in 1992, employing zidovudine (ZDV) monotherapy. The national ART programme was providing free ART for HIV/AIDS patients who were covered by the universal health insurance scheme of the Ministry of Public Health. Patients participating in clinical trials or university research projects, or qualifying for the Social Security Fund Scheme, also obtained free treatment. Both treatment-naïve and treatment-experienced patients were being enrolled on the basis of evidence of clinical AIDS and/or CD4 measurement. Effective, affordable and manageable drug regimens had been defined, the more costly regimens having been reserved for second-line and third-line therapy. Specific training programmes for ART had been implemented. Protocols for the enrolment and management of children had been developed. The planning of associated logistics, including the procurement and supply of drugs, laboratory diagnostics and equipment, and information technology and data collection, was well developed. During 2004, recruitment to the programme had been increased, over 3000 new persons having entered it each month and over 900 hospitals providing ART services. It was expected that, by the end of 2004, approximately 50 000 patients would be receiving ART. A target of 80 000 on ART by the end of 2005 had been set.

d. Brazil

The situation in Brazil possibly differed somewhat from that in other countries affected by the HIV epidemic, since Brazil was a middle-income country with an annual per capita gross domestic product of US\$ 2998 and a life expectancy at birth of 68 years.

In mid 90's the Brazilian Government began to offer free ARV drugs to all citizens infected with HIV, on the basis of criteria set by an independent committee. The treatment guidelines were more conservative than those in the USA, e.g. they were CD4-guided, therapy was initiated later and dual therapy regimens were accepted (triple therapy became available later). By the end of 2002 there were 130 000 people on ARV drugs and, by the end of 2004, 305 hospitals, 73 day clinics and 166 special HIV units had been accredited for HIV care.

In order to support the national HIV programme, Brazil invested heavily in laboratories that could perform all HIV-related testing, including both CD4 counts and VL testing. At the time of the consultation there were 70 CD4 laboratories and 65 laboratories performing VL testing throughout the country in the public health sector, and many more in the private sector.

The laboratory support programme and ART itself were not instant successes. In 1997, only 28% of patients on ART had VL < 400 copies/ml after six months on treatment. By 2004, however, 98% of patients had VL < 400 copies/ml after six months. The laboratory programme had also matured over that period. In 1996, five laboratory specialists visited HIV laboratories in Canada and returned to establish the first laboratories dedicated to HIV. This group evolved into a committee that was still meeting regularly. The major areas of focus were no longer on scale-up but on limiting the attrition of trained technologists, who often left for higher-paying positions elsewhere, and on formalizing the QA programme. Quality management (QM) had been an important part of Brazil's laboratory support programme. External quality assurance for CD4 was conducted six times per year, three in a national programme and three in an international programme, so that each laboratory was assessed every two months. In the public health sector, few tests were done in real time and there were significant delays in the reporting back of results to the clinical sites. For people who could afford it there was also a well-established private health sector. In answer to the question as to whether the building of capacity or the rolling out of programmes should come first, Brazil had shown that both could be done at the same time.

e. Médecins Sans Frontières

Médecins Sans Frontières (MSF) was providing care and treatment to 23 000 patients at 27 project sites in 23 countries. In general, CD4 measurements were used as a tool for starting treatment (baseline) and then for monitoring the efficacy of the treatment regimen every six months. Total lymphocyte counts were not used as they were not felt to be sufficiently reliable. The equipment used in the MSF programmes included Becton-Dickinson's FACSCount (15 programmes), Dynal's Dynabeads (five programmes) and Partec's CyFlow (four programmes). In Malawi (Chiradzulu) and Mozambique (Tete and Angonia), patients were enrolled on the basis of WHO clinical stages 3 and 4 without the use of CD4 baseline data.

Four of the 27 MSF projects were measuring VL (Cameroon, Guatemala, South Africa and Thailand) by means of either Amplicor (Roche) or NASBA (bioMérieux). The major questions confronting MSF as it considered whether to pursue VL monitoring in its projects were as follows.

- (1) Was individual monitoring of VL a viable option?
- (2) What was the role of sentinel surveillance, and what VL systems would be most useful?
- (3) Would new, practical technologies for VL measurement be available soon, e.g. dipstick, dried blood spots?

In MSF projects monitoring VL the treatment results had been consistent with other reported results in low-income countries. Thus a cross-sectional VL survey in Malawi showed that, after six months of treatment, 73% of 477 patients had VL < 40 copies/ml, 85% had VL < 400 copies/ml and 88% had VL < 1000 copies/ml. After two years, in a limited group, 14% of patients had VL > 5000 copies/ml in two consecutive determinations.

MSF was still not sure how best to use VL. Initially, VL was used as an advocacy tool for demonstrating that ART was feasible in resource-poor settings. Staff in South Africa used VL to assess adherence but were often reluctant to change a treatment solely on basis of VL results. Operational research was necessary in order to determine how VL could be used to decide when switching to an alternative regimen was required with a view to improving patient outcomes.

Few tests were available for diagnosing OIs. MSF projects could reliably detect and diagnose only TB and *Cryptococcus*. There were no affordable tests for diagnosing cytomegalovirus, *Pneumocystis carinii* (PCP) and other major OIs. These tests were at least as essential to HIV care as VL measurements. Strategic approaches involving decentralization and/or centralized testing were presented. Where logistical constraints could be overcome and the quality of specimens could be preserved, blood specimens could travel instead of patients.

III. Clinical monitoring issues

a. Clinical monitoring of adults

Monitoring the efficacy of ART included observations on clinical, immunological and virological parameters. In the first six months, clinical monitoring could be difficult because of the continuation of OIs, immune reconstitution inflammatory syndrome and the appearance of side-effects of the drugs. It was necessary that algorithms for diagnosing treatment failure with limited laboratory support be based on treatment history, clinical manifestations, adherence information and simple laboratory tests. It was felt that adherence history and the evaluation of adherence through self-reporting by patients, the use of a visual analogue scale and treatment cards were not being fully utilized.

It was necessary to address the question of VL monitoring in the context of clinical decision-making. In general, VL should be regularly measured and if so, values need to be established as a trigger for determining when to switch from first-line to second-line therapy. It was worth considering whether alternative data points existed, e.g. clinical parameters, laboratory test results or other patient information, which might be used to reduce dependence on expensive VL testing in guiding clinical decisions.

As a general rule it was desirable that physicians, before ordering expensive tests, should consider the use to be made of the results and the treatment options available for the patient.

b. Clinical monitoring of children

The Botswana programme was triggered by a survey in 2001 which revealed that there were more than 300 000 HIV-infected people in the country and that 100 000 individuals were eligible for ART, of whom over 90% did not know their HIV status. Among the many reasons for this hidden epidemic were substantial sociocultural barriers and an almost total lack of laboratory capacity.

Several groups in Botswana began collaborating to establish comprehensive HIV care. In December 2001 the first HIV laboratory opened, staffed by a team from the Botswana-Harvard Partnership. This sophisticated facility offered CD4 and VL testing, HIV serology and basic haematology and chemistry. Resistance testing was added in 2004.

The initial laboratory monitoring protocol in Botswana mirrored that of high-income countries. Patients underwent baseline CD4 and VL testing, and were then tested for CD4 and VL every three months. Full blood counts were obtained after one month on treatment and at three-monthly intervals thereafter. Patients taking nevirapine (NVP) underwent regular liver enzyme testing. Lipid levels were measured every six months in patients taking protease inhibitors.

The Botswana-Baylor Children's Clinical Centre of Excellence in Gaborone had more than 1200 paediatric patients. The clinical monitoring of children on ART covered:

- (1) growth (weight and height measured every three months);
- (2) development (occipitofrontal circumference measured every 3 months for children aged under 2 years, and neurodevelopmental assessment made every 3 months);
- (3) social well-being (access to social benefits, school attendance and quality of care by carers);
- (4) immunizations.

The Botswana roll-out encountered numerous problems, including a lack of trained pharmacists, poor stock management, poor linkages between HIV programmes and the need to integrate HIV programming into the general health system. With respect to laboratory services, the major problems were with centralized testing at a single site, including difficulties in transporting samples. A significant investment in the laboratory infrastructure had been critical in accelerating the pace of roll-out. It was necessary to make the system sustainable.

Discussion

There was general agreement that it was necessary to give more attention to standardized clinical patient monitoring by means of a set questionnaire and to the monitoring of adherence. Although the potential role of VL testing in the decision-making process for switching to second-line treatment was debated, no data were available on which to base clear recommendations. The lack of appropriate diagnostic tests for early detection of HIV infection in infants born to HIV-positive mothers (qualitative HIV DNA/RNA tests and/or p24 Ag test) and for monitoring infants and CD4 %, and easy specimen collection and transport (filter paper) was identified as a gap that urgently need to the addressed.

IV. Laboratory technologies and monitoring

a. Resistance monitoring

The accelerated roll-out of ART could lead to the emergence and transmission of ARV-resistant viruses. Although HIV drug resistance (HIVDR) could not be prevented, there might be a possibility of combating its spread and impact. HIVDR developed as a result of the high replication rate of HIV and its error-prone transcription. ART, particularly if suboptimal, selected mutations that could replicate in the presence of ARV drugs, and virological failure could rapidly emerge.

WHO had prioritized the prevention, surveillance and monitoring of HIVDR within its planning for the 3 x 5 initiative and had developed an approach to preventing the emergence and transmission of HIVDR. This approach included the use of standard ART regimens that were highly active and suited to the countries or regions concerned. Standard individual treatment records and active monitoring of adherence were being established. Measures to ensure the continued supply of drugs of satisfactory quality were essential to the success of the initiative, as were programmes for people receiving ART in order to reduce HIV transmission. The establishment of HIVDR surveillance and monitoring would provide a means of assessing whether implementation had been successful. HIVDR surveys would target untreated individuals in specific geographical settings who had recently become HIV-infected. WHO intended to evaluate whether HIVDR was < 5%, 5–15% or > 15% in various regions by examining sufficient specimens to provide adequate statistical power for a threshold survey. Public health action would be essential if such a survey indicated HIVDR to be > 15%.

Monitoring would allow evaluation of the patterns of drug resistance emerging in sentinel centres with first-line regimens. It was planned to check for HIVDR before the initiation of ART, at 12 and 24 months, and before changing to second-line ART. This would provide data on the appropriateness of first-line ART, patterns of HIVDR associated with specific ART regimens at different sites, the ability to monitor the appropriateness of second-line ART, and validation of the HIVDR early warning measures. Standard laboratory protocols for the detection of HIVDR would be applied and QA measures would be strengthened. The findings would be assembled in national and regional databases and it was intended that regional and global HIVDR reports would be prepared twice a year.

b. Technologies for resistance monitoring

There were two basic approaches to testing for the presence of HIVDR: phenotyping and genotyping.

Phenotyping measured the ability of the virus to infect or replicate in vitro in the presence of drugs. Because of problems inherent in the isolation and culture of HIV from individuals under investigation, an approach using recombination to insert an amplified region from the *pol* gene into a plasmid HIV was usually employed. This recombinant was used in a cell culture system to assess the phenotypic susceptibility of the originator virus against a range of drugs. Although expensive and time-consuming, this method provided a relatively direct measure of susceptibility, assessed the effect of all mutations, even those not yet described, and could easily be adapted to test new classes of drugs.

Genotyping provided direct information on mutations at codon positions of (RT) and protease genes associated with drug resistance. Because it relied on polymerase chain reaction (PCR) amplification of all the quasi species present in an individual, population-based sequencing generated a consensus sequence that would identify only the major species present. However, the generated sequence would provide additional information, including the HIV subtype/ circulating recombinant form (CRF) and information on possible cross-contamination. Point mutation assays would detect only the particular set of mutations designed into the assays but might identify minority sequence populations. Genotypic methods were more rapid, cheaper (but still very expensive) and did not require the biosafety level 3 precautions needed for phenotyping. However, they required expert interpretation of drug resistance algorithms, and these needed regular updating for new drugs or combinations of drugs. Moreover, the presence of naturally occurring polymorphisms in HIV-1 non-B viruses at codons associated with resistance in HIV-1 clade B viruses further complicated genotypic resistance testing. Consequently, research was needed to develop reliable genotypic interpretation algorithms for non-B HIV-1 strains and to study mutations selected in non-B strains. Performance assessment programmes for laboratories providing HIVDR testing were essential and had to be further developed and expanded.

c. CD4 technologies

The latest information on CD4+ T-cell enumeration technology was reviewed, with particular reference to resource-limited settings. The following trends in flow cytometry were leading towards low-cost monitoring tools:

- (1) smaller instruments;
- (2) fewer reagents;
- (3) digital image analysis;
- (4) improved sample stability;
- (5) wireless data transmission for quality control.

One of the first tasks in the development of CD4 technology for resource-limited settings was to define the specifications of the instrument and the estimated workload, in order to ensure an appropriate choice of technology. The following important questions had to be considered.

- ▶ Would the technology be used to measure CD4+ T-cell counts in adults, children or both?
- ▶ Would it be used in a rural setting or an urban setting?
- ▶ Would it be used in a central referral centre, a district hospital or a peripheral health care site?
- ▶ Would testing be done at the point of clinical service or would blood be shipped from the clinical site to a nearby or distant laboratory? (Advances in CD4 technology make it possible to consider whether to bring the laboratory to the patient or vice versa.)

High-end flow cytometers (e.g. the Becton-Dickinson FACSCalibur and the Beckman Coulter EPICS XL) were still being used in low-income countries, primarily in major cities with well-developed central laboratories. In order to meet the needs of ART scale-up, however, these high-end, expensive and technically more complex high-throughput machines would not be useful in more remote settings, including most district hospitals with limited financial and human resources. In these settings, less expensive and simpler equipment and techniques were needed.

At least two companies, Partec and Guava Technologies, were producing middle-end flow cytometers designed for use in resource-limited settings. Their technologies were being evaluated.

The Partec CyFlow Counter was a self-contained flow cytometer with one parameter, while the same firm's Cyflow Green had two parameters. Both of these instruments could run on a car battery. More powerful, build-on-order flow cytometers were obtainable from Partec. Field experience had shown that the CyFlow systems might not have overcome technical challenges related to the robustness of the equipment or the reproducibility of the results. The Cyflow Counter was possibly inappropriate for TB-coinfected patients.

The EasyCD4 system of Guava Technologies used a less expensive light source and coupled the cytometer to a laptop computer for analysis. Prices per test could be reduced to \$1.00–2.00 because the method involved using low volumes of reagents and did not need sheath fluid. The instrument was undergoing independent investigation by the Centers for Disease Control and Prevention (USA) and WHO in order to determine its robustness and accuracy in the field. Laboratory technicians required a sufficient understanding of flow cytometry in order to be able to operate these middle-end instruments reliably and make adjustments if necessary.

The following three distinct approaches were currently available for measuring CD4 T-cells in resource-limited settings where the throughput was low, i.e. fewer than 30 specimens/day.

- (1) **Automated equipment** could be used which produced a CD4 T-cell result without significant technical expertise or interpretation on the part of the user. Two systems meeting the criteria were available, only one of which, the Becton-Dickinson FACSCount, had been widely adopted. This system used proprietary reagents to produce an absolute CD4 T-cell count from a single 1-ml sample of whole blood. It measured CD3, CD4 and CD8 cells, but had not been configured to measure CD4 T-cells as lymphocyte percentages. Consequently, the FACSCount system was only useful for CD4 T-cell counting in adults. The company had been asked to alter the parameters so as to allow CD4 percentage determinations for paediatric use.

In 2004, PointCare Technologies introduced the FlowCare system of CD4 measurement. The product was being marketed by Beckman Coulter under the name of PointCare. The approach differed slightly from that of traditional flow cytometry. It used exclusively light-scattering parameters to distinguish

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lymphocytes from other white blood cells. CD4 cells labelled with a colloidal gold-coupled antibody were then identified on the basis of wide-angle light scatter. The net result was that the entire system was automated as from the point of blood collection. A whole blood sample of 3–5 ml was placed in the machine, and after single-button operation a CD4 result was reported in approximately 17 minutes. PointCare provided CD4 percentages and absolute counts, total white blood cell counts, total lymphocyte counts and lymphocyte percentages.

The company was currently finalizing some modifications of methods which would soon allow the system to deal with samples from both adults and children. However, as PointCare used a different anti-CD4 antibody-labelling method there were no generic reagents available, and the system was not compatible with existing EQA programmes.

- (2) The following **manual methods** could be used. They had a lower throughput, i.e. fewer than six specimens/day, but were less expensive overall. Both employed microbeads for the identification of CD4 T-cells. Both systems provided only absolute CD4 counts.
- (a) Dynal's Dynabead system used two immunomagnetic separation steps to (i) remove CD4 monocytes and (ii) isolate CD4 lymphocytes, which could then be counted under a light or fluorescence microscope.
 - (b) Coulter's Cytosphere method blocked the CD4 receptors on monocytes with one set of microbeads and labelled the remaining CD4-positive cells with a larger set of colour-labelled beads. These rosetting cells could be distinguished under a light microscope.

The principal problems concerned the time involved in processing and analysing the specimens and the throughput, as the two-step cell isolation and microscopic counting process took a single technician up to one hour per patient result. No EQA programme was available for these manual techniques.

- (3) **Whole blood** specimens from patients could be **stabilized** and transported to a central laboratory with high-end equipment. Four blood stabilizers were on the market: Cyto-Chex BCT (Streck Laboratories), Cellsave (Immunocon) (R&D Systems), ThromboFix (Beckman Coulter) and TransFix (UKNEQAS). These blood fixatives all appeared to stabilize blood reliably for up to seven days at 22°C but only with Transfix were specimens stable for three days at 37°C. Unfortunately, temperatures above 37°C were common in many parts of the world where HIV was prevalent.

LabNow was developing a point-of-care CD4 T-cell counting instrument based on microfluidic sample processing and digital image analysis. It would possibly be battery-operated and portable, thus providing a useful approach to CD4 counting in resource-limited settings. It was anticipated that the product would be available for validation in the second half of 2005 and that, if all went well, it would be ready for wide-scale use in 2006.

Other assays under development for use near the point of care included various dipstick approaches and a cell capture assay involving microscopy (Sembio).

Attention was repeatedly drawn to the lack of affordable instruments that could produce a CD4 percentage of total lymphocytes, which was desirable for monitoring infants and children aged up to 6 years. To date, only high-end flow cytometry equipment could produce reliable CD4 percentages. The FACSCount system had not been configured for paediatric use, although appropriate reconfiguration would be feasible. PointCare provided CD4 T-cell percentages but had not yet been validated in respect of CD4 percentage assays in children. The absence of affordable paediatric CD4 counting technologies was a huge barrier to ART roll-out in children. The development of such technologies was of the utmost importance.

Discussion

Several middle-end and low-end CD4 technologies had recently been commercialized or would soon become available. Although several of the instruments seemed very promising, it was necessary to assess their performance with specimens from both adults and children in independent studies. Negotiations with and competition between manufacturers of CD4 technologies were resulting in price reductions. It was desirable to organize a survey that would give a better view of the operational aspects and down time of CD4 equipment at the country level. It was emphasized that local maintenance expertise was crucial for reducing the down time of the instruments.

d. Quality assurance

The activities managed from the Australian National Serological Reference Laboratory (NRL) were used as a model in describing the elements of QA. In addition to organizing EQA and producing quality control (QC) samples for Australia, NRL had provided, in collaboration with WHO/EHT, similar services to many countries of the South-East Asia Region and the Western Pacific Region. The WHO EQA and QC activities had been supported by an active programme of training and other support through workshops, published guidelines and advice. It was essential to have mechanisms in place for ensuring the availability and use of kits of high integrity, monitoring continued quality from batch to batch, and ensuring their correct use. Error rates in the WHO HIV / hepatitis B / hepatitis C serology EQA schemes run by NRL in the South-East Asia Region and the Western Pacific Region had fallen, presumably as a result of the consistent application of quality activities. EQA for HIV-1 VL measurements had shown that performance in Thai laboratories was similar to that in Australia. A web-based EQA system called Electronic Data Collection was a valuable tool permitting real-time monitoring both of laboratory performance and the various diagnostic kits in use. A similar approach had been followed by WHO/EHT for providing a QA programme supporting CD4 T-cell counting in the African and Asian regions. WHO was working closely with the Canadian EQA scheme for CD4 and had established a network of centres of excellence in the regions to provide EQA for CD4 T-cell counting, training and technical support.

e. HIV incidence testing

The ability to discriminate between long-standing and recently acquired or incident HIV infection was important, providing insight into current transmission trends within epidemiological surveys, aiding the tracking of outbreaks and possibly assisting in the monitoring of treatment programmes. Several techniques had been described, including STARHS (serologic testing algorithm to detect recent seroconversion or detuned) IgG capture assay (BED assay) and antibody avidity assays. NRL had also researched methods of detecting incident HIV infection and had identified a new method that tested for the IgG3 isotype of HIV antibody, which seemed to appear for a period of several months after seroconversion. Additional work was needed on tests for incident infection so as to improve the identification of thresholds and window periods and their specificity and sensitivity. How they performed when applied to individuals with non-B subtype infections also required further elucidation.

f. Relevant laboratory technologies in antiretroviral therapy roll-out programmes

Several countries had progressed significantly with national ART roll-outs, and important lessons could be learnt on the implementation of appropriate diagnostic and laboratory monitoring systems. In a very short time South Africa had rolled out its ART programme and scaled up its laboratory capacity. Many problems had been solved but some remained.

For **haematology** it was important for the national laboratory system to recognize that the method and approach to full blood counting was driven by the choice of CD4 technologies. Many systems were still of the dual platform kind and required a lymphocyte differential from a full blood count in order to back-calculate an absolute CD4 count. The choice of a single-platform as opposed to a dual-platform CD4 counting system therefore had significant implications for the haematology analysers that could be used.

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Moreover, the values being used as reference ranges were based on published data, most of which were obtained from studies in high-income Western countries. Little was known about the normal reference range for white blood cell counts and subsets in much of sub-Saharan Africa and South-East Asia. This information could be critically important as widespread CD4 counting became available and CD4 counts began to be used for decisions about expensive treatments. However, even if normal ranges varied there were no data to suggest different trigger points (e.g. CD4 < 200, or CD4% < 15%) for the initiation of treatment.

The challenges associated with **chemistry** results were even more significant. The most pressing problem was that nearly all currently manufactured automated chemistry systems were high-end expensive machines bundling chemistry assays in one device. It could thus be difficult to obtain a single result for a liver enzyme, e.g. alanine aminotransferase (ALT), at low cost with less complex equipment. There were ways of obtaining these single chemistry results at lower cost but they were not generally automated and involved low throughput.

Perhaps the biggest challenge facing **national laboratory programmes** was the need to handle extremely high volumes of **CD4 testing**. In South Africa the national programme had had to expand from 3 to 22 laboratories capable of performing CD4 counts in less than a year. It could be difficult, but not impossible with good planning, to maintain an effective programme that met QA standards. Analysis of the South African data had shown that CD4 counts below 200 cells/mm³ had been observed in more than 50% of patient tests.

In South Africa, regular **VL** measurements were part of the national protocol. This had led to extremely high volumes of VL testing, which had also been scaled up from a few to many laboratories. It was necessary to consider carefully the volume of testing when deciding what equipment to use. Systems relying heavily on manual nucleic acid extraction at the front end, including the bioMérieux system used in South Africa, began to struggle if volumes were high. Automated sample extraction was likely to be more appropriate for high-volume settings.

In addition to these specific concerns there were general challenges to laboratory scale-up. Although there was a tendency to focus on CD4 and VL, many laboratories in

resource-limited settings still struggled with basic tests involving HIV serology, basic haematology and basic chemistry.

QM and QA systems were essential for the achievement of accurate laboratory results. These systems could be rapidly introduced in resource-limited settings by using existing EQA programmes for assistance. A training plan was essential for implementing laboratory scale-up. Technicians and staff had to be trained in good laboratory practice (GLP) and the use of specific equipment. Laboratory site initiation modules could support GLP, standard operating procedures (SOPs), QM systems and specific training.

It was necessary for laboratory operational plans to be prepared for the enormous volumes that were likely to accompany national roll-out, particularly for CD4 testing, which were used for both patient staging and patient monitoring.

Infant diagnostics were still being neglected. There was no validated low-cost method for diagnosing infection in children aged under 15–18 months, yet this was a vital requirement, especially for programmes concerning transmission between mothers and their children.

Laboratory information systems were still unlinked to clinical information systems, making communication between laboratories and clinics both more critical and more difficult.

g. HIV/tuberculosis laboratory issues

It was estimated that 10–30% of HIV-infected people had TB. In certain regions more than 50% of TB patients were HIV-positive. Because of the scope of HIV/TB coinfection it was necessary for HIV ART roll-out programmes to take this into consideration. It was essential for HIV and TB programmes to collaborate closely, particularly with regard to laboratory matters. Both at the case-finding level and the ARV entry-point level, it was desirable for HIV diagnosis to trigger an algorithm for TB case-finding, and vice versa. The tests were carried out in the same laboratory, sometimes even by the same technician. Consequently, it was desirable for there to be more integration and the building of linkages and referral mechanisms between the HIV and TB laboratory programmes, including cross-checks of HIV and TB laboratory registers, the strengthening of QA, joint laboratory supervision and overall monitoring.

There were significant limitations on the availability of appropriate and easy-to-use diagnostics for TB. The accuracy of the smear test was declining because of HIV coinfection. Furthermore, the percentage of extrapulmonary TB was increasing and X-ray facilities were therefore required. However, they were not always available. TB diagnosis by culture was relatively costly, complex and time-consuming and required an appropriate laboratory infrastructure.

New TB diagnostics were being assessed but none seemed to be working satisfactorily. New approaches and technologies were urgently needed for identifying latent TB infection in a rapid and inexpensive manner.

Discussion

It became apparent that there were several overarching issues related to laboratory monitoring. In resource-limited settings with low throughput there was a requirement for appropriate, reliable, easy-to-use and inexpensive laboratory technology for haematology, chemistry, CD4 counting, VL testing and TB diagnostics. Another recurrent issue was the need for QA and training, particularly when new technologies were being introduced and in low-throughput settings, i.e. in circumstances associated with roll-out programmes. Integration of the different laboratory components would be vital to the success of 3 x 5 programmes. National laboratory committees would have to demand and actively support the roll-out of ART programmes.

V. Prequalification and procurement issues

a. UN prequalification process for antiretroviral drugs

It was pointed out that millions of people living with HIV/AIDS did not have access to treatment. Substandard medicines and counterfeit products were procured and supplied in various countries. QA systems in medicine supply chains were often weak or did not exist. The sourcing of products of poor quality presented risks to patients by increasing treatment failure and resistance.

The objective of the project was to propose a list of prequalified manufacturers and products whose quality, efficacy and safety had been assessed, inspected and controlled so that they met the international norms and standards.

WHO managed the prequalification project on behalf of the UN. Its main partners were UNAIDS, UNFPA, UNICEF and the World Bank.

Prequalification involved a standard procedure developed by the WHO Expert Committee on Specifications for Pharmaceutical Preparations. The assessment of product dossiers submitted by companies and inspections on good manufacturing practice were followed promptly by feedback to the companies concerned. Medicines were added to the list of prequalified products only when the products and manufacturing sites met the required standards. The list of HIV-related products and manufacturers which were found to be acceptable in principle was available on the web sites of collaborating UN agencies.

WHO provided the technical and scientific expertise and guarantees that international norms and standards were applied throughout the process, including the assessment of the dossiers, inspection and quality control.

The submitted dossiers were reviewed by a team of eight to twelve experts in order to check their acceptability with respect to quality and efficacy. Additional data could be requested. For reasons of objectivity, each dossier was assessed by two experts and an assessment report was written and made available. The second step was the inspection of the manufacturing site or sites and the inspection of the research laboratory where the bioequivalence study was performed. The QC programme was also assessed and products were requalified after three years.

The experts involved in this process were mainly assessors and inspectors from national drug regulatory authorities or national QC laboratories of Member countries of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.

In December 2004, only 85 HIV-related medicines, 8 antituberculosis drugs and 2 antimalarial drugs had been prequalified out of a total of 500 products for which dossiers had been submitted. It was necessary to develop the prequalification project further so as to cope with the increasing number of dossiers, the follow-up of variations, and periodic requalification, and to extend its assistance to both manufacturers and the national drug regulatory authorities in resource-constrained countries.

b. UN assessment and selection of appropriate diagnostic technologies

WHO, on behalf of the UN family, provided technical advice to Member States on the quality and operational characteristics of diagnostics for HIV care and treatment. As part of this effort, several diagnostic instruments and assays had already been assessed or were scheduled for evaluation.

In general the evaluation should proceed along the following steps:

- (1) WHO should receive a request from a manufacturer or the request should be initiated by WHO.
- (2) WHO should review independent data on performance generated in trials and review existing certifications, e.g. FDA approval or the CE mark.
- (3) If not the manufacturer but a third party requests the evaluation, an OEM investigation should be pursued. An original equipment manufacturer investigation should be conducted into the repackaging of an assay sourced from a single manufacturer under a different label which did not involve alterations in the production of the assay components.
- (4) A letter of agreement should be signed between WHO and the manufacturer or third party.
- (5) The operational characteristics and the assay and/or equipment performance should be evaluated. Assay and/or equipment performance should be evaluated at WHO collaborating centres in various part of the world.
- (6) The data from each stage of the evaluation should be analysed and the information should be disseminated.

The evaluation of **HIV serological assays** relied on a well-characterized WHO reference panel of specimens and a standardized algorithm. Minimal performance criteria had been established with regard to sensitivity (> 99% for rapid, 100% for enzyme-linked immunosorbent assay (ELISA), specificity (> 98%) inter-reader variability (< 4%) and seroconversion sensitivity. The clarity of the test kit insert, the labelling of the component of the kit and the easiness of the test procedure were also assessed.

The validation of **CD4 technologies** was conducted at several sites in accordance with one protocol. Specimens were collected in a prospective manner and a certain number of control specimens were included in each

assessment. The results were compared to those obtained by gold standard methods. The performance criteria included accuracy, linearity, reproducibility/precision, inter/intra-run variability and technician variability. The Dynabeads, Cytospheres, FACSCount and a few assays no longer available had previously been assessed by WHO. Several performance assessments were in progress, covering CyFlow Counter, Cyflow Green, EasyCD4 and PointCare. Contacts had been made with the manufacturers of FlowCare and Sembio with a view to assessing their technologies as soon as they became commercially available.

The assessment of VL assays covered sensitivity to the various HIV subtypes, reproducibility/precision and accuracy. The data were compared to those obtained by gold standard methods for these assays. The ExaVir v2.0 (Cavidi®) and the Retina Rainbow assay (Primagen®) were currently under evaluation.

WHO was concerned mainly with diagnostics and equipment appropriate for use in resource-limited settings. In many of the countries heavily affected by the HIV epidemic, the regulatory capacity for diagnostics was weak or non-existent. WHO could ease the work of the national regulatory authorities by providing technical performance data as well as criteria that were not related to performance, e.g. ease of use and the appropriate level of health service for use, e.g. VCT centre versus district versus regional. Manufacturers of assays that had been successfully evaluated were eligible to tender for bulk procurement through the UN. The one-year procurement agreements between companies and WHO covered the UN family (UNAIDS, UNDP, UNFPA, UNICEF, World Bank, etc.). Access to diagnostics of satisfactory quality at reasonable cost was thus made available to Member States.

Prequalification and procurement issues

c. WHO Contract Procurement Services

WHO was directly involved in the procurement of laboratory equipment and test kits. Orders could be placed through WHO by Member States, nongovernmental organizations (NGOs) in official relation with WHO, and members of the UN family. A new electronic catalogue and ordering system, WebBuy, was now on line and accessible in the offices of WHO country Representatives. The on-line catalogue made simplified bulk procurement possible for Member countries. Currently, ten catalogues were available, including one devoted to HIV laboratory materials which provided information to potential buyers. A purchase order system, facilitating the whole process, was linked to the catalogue. The WHO bulk procurement scheme for HIV diagnostics, established in 1989, was continuing to evolve and to adapt to current needs.

d. UNICEF Procurement Services

UNICEF had established a supplies procurement system that worked directly with country offices, other UN agencies and NGOs. There was a UNICEF warehouse in Copenhagen where commodities could be stored if necessary. It could serve as a link to bulk procurement for the end user. ARV drugs, HIV test kits and CD4 and VL equipment valued at \$18 million were procured during 2004 through the UNICEF Supply Division; 26% of the procurement had been in Ghana, 21% in Malawi, 14% in Myanmar, 7% in the Democratic Republic of the Congo, 7% in Zambia and 5% in Cambodia. It was difficult to forecast demand as good systems for data collection were not yet in place. Quantities of HIV test kits were small and mainly for new customers, resulting in classic start-up problems. Among other problems linked to scale-up were the frequent disregard of the costs of in-country distribution in plans and a lack of knowledge of appropriate laboratory infrastructures and general laboratory commodities. The Supply Division developed survey questionnaires and equipment planning bulletins for HIV/AIDS service providers, with a view to facilitating planning at the primary and first-referral levels.

e. AIDS Medicines and Diagnostics Services

AIDS Medicines and Diagnostics Services (AMDS) was a network supporting procurement and supply management for HIV drugs and diagnostics. Members of the network included WHO (EDM, EHT, CPS, regional offices), UNICEF, the World Bank, UNAIDS, UNDP, UNFPA, the Centre for Collaborative AIDS Research, the Clinton HIV/AIDS initiative, the Commonwealth Pharmaceutical Association, the Crown Agents, the Ecumenical Pharmaceutical Network, Esther, the Global Fund for AIDS, Tuberculosis and Malaria (GFATM), IDA, the International Pharmaceutical Federation, John Snow Incorporated and Management Sciences for Health. AMDS served as a clearing house for information and as a broker for assistance to country partners for bulk procurement. It could provide forecasting tools and supply management tools to enable centralized planning of bulk procurement.

f. Global Fund for AIDS, Tuberculosis and Malaria

The contributions paid to GFATM in 2004 amounted to \$1446 million. A large number of proposals had been received and funded. The Board anticipated that a request for Round 5 proposals would be launched by mid-2005.

The procurement of ARV drugs and HIV/AIDS-related diagnostics constituted a substantial component of most of the proposals. GFATM was not being prescriptive on the types of products or their suppliers. Countries were free to order any products from any supplier at any given cost. However, GFATM acknowledged the UN prequalification project for priority medicines, and countries were encouraged to buy prequalified products. In many countries, relatively weak procurement and supply management systems had hampered the implementation of accepted proposals. Consequently, the beneficial aspects of working with well-experienced supply agencies had been recognized.

Discussion

Some concerns were raised about the duplication of efforts in procurement and supply management within WHO and between UN agencies. It was explained that there was good collaboration between WHO and UNICEF as well as with the other UN agencies. WHO was the lead agency on technical issues such as the prequalification of ARV drugs (EDM) and on the validation and selection of HIV/AIDS-related diagnostics (EHT). The AMDS web pages provided a one-stop shop for the technical information relevant to countries and partners.

The advantages of the existing UN procurement systems were that countries did not need to go through a lengthy tendering process and had access to products of satisfactory quality at reasonable cost not linked to volume. However, both shortages and wastage of diagnostics occurred because forecasting was difficult in a scale-up situation. Stock management systems were required with regular feedback to the central purchase point so as to ensure continuous access to ARV drugs and diagnostics.

VI. Group work and recommendations

Group work 1. Review of current laboratory requirements supporting antiretroviral therapy roll-out: clinical experts

The clinical expert team comprised two groups (A and B) and the major issue was to review and strengthen the basic laboratory monitoring recommendations previously established on pages 24-26 of the current WHO ART guidelines (*Scaling up ART in resource-limited settings – 2003 revision*). With regard principally to Table E (*Recommended tiered laboratory capabilities for ARV monitoring in limited-resource settings*) and Table F (*Basic laboratory monitoring for WHO-recommended first-line regimens at community health and district hospital centres*) of these guidelines, the basic questions for debate were as follows.

- 1) What tests were needed?
- 2) When (what frequency)?
- 3) Where (i.e. at which level of care, e.g. tertiary hospital, district hospital, health centre)?

Recommendations of Group A

Group A was in general agreement with the content of Table E but emphasized that clinical and laboratory investigation was essential for the diagnosis of TB at all levels and that the link between TB and HIV programmes had to be strengthened. It was suggested that any HIV antibody testing (not restricted to rapid test methodologies) should be considered for HIV diagnosis at all levels.

Regarding Table F, it was recommended that a more detailed frequency schedule for monitoring the suggested tests should be established, particularly for regimens based on ZDV and NVP. For patients to be considered for the use of ZDV, it was recommended that there should be an Hb monitoring schedule at baseline, followed by monitoring at 4, 8 and 12 weeks during the first 3 months, and, thereafter, in accordance with symptoms. For NVP users the recommendation was for baseline CD4 and ALT measurements before the initiation of treatment, ALT measurements at 4, 8 and 12 weeks, and symptom- directed monitoring thereafter, if these tests were available.

It was suggested that a specific table for second-line drugs was needed and that, for TDF users, creatinine and urine protein should be checked. For patients considering the use of lopinavir/r it was desirable to monitor blood glucose and lipids if possible. A symptom-directed investigation for pancreatic toxicity was recommended for users of ddI. No specific laboratory test was recommended for abacavir.

Regarding laboratory efficacy monitoring, Group A suggested that the CD4+ cell count should be evaluated every 3–6 months if possible. There was a long debate about VL but no agreement was reached on a definition of virological failure. Sequential measurements, rather than a single result, were more useful in monitoring the virological response. Some comments and questions for future evaluation were highlighted: the potential existed for using the 6-month VL measurement to detect early adherence problems, and it was important not to create obstacles to scale-up for countries with no capacity to perform VL testing. For countries with this capacity, however, specific guidelines were needed.

Recommendations of Group B

Although Group B highlighted the importance of the physical environment (laboratory safety, biohazards), it was emphasized that this consideration should not hamper efforts to scale up laboratory capacity. It was felt that the investment in laboratory services should be based on volume and prevalence and that clinical capabilities should be enhanced accordingly, taking into account the learning curve.

Group B considered that the HIV antibody test was the only test that was invariably essential at the primary care level, but that Hb and pregnancy tests were desirable and that the sputum smear test for TB evaluation should be referred if microscopy was not locally available.

It was considered that, at the district hospital level, the following tests should be available for monitoring ART: HIV test, full blood count (FBC), differential blood count, pregnancy test, sputum smear test for TB, second HIV serological method (to confirm HIV antibody test results), CD4+ cell count, ALT and possibilities for the diagnosis of treatable OIs (e.g. cryptococcosis, toxoplasmosis, PCP) in accordance with clinical capabilities.

It was suggested that, at the regional level, full serum chemistries be added if available, including but not restricted to electrolytes, renal function, liver enzymes and lipids. VL testing was desirable but not essential at this level.

The following recommendations were made on the frequency of testing.

- ▶ HIV antibody testing (confirmed result): baseline (once).
- ▶ Hg measurement: baseline, 1 and 3 months, then symptom-directed or if clinically indicated
- ▶ Pregnancy test: if indicated.
- ▶ Sputum smear test for TB: if indicated.
- ▶ ALT: if indicated.
- ▶ FBC and differential: baseline, 1 and 3 months, and then symptom-directed or if clinically indicated
- ▶ CD4+ cell count: baseline and every 6 months (if available at district level and recommended at regional level).
- ▶ Diagnosis of treatable OIs: if indicated.
- ▶ Full serum chemistries: baseline and every 6 months.
- ▶ VL: if indicated.

Tables 1–3 harmonize and clarify the recommendations made by Group A and Group B.

Group work and recommendations

Table 1

Recommended tiered laboratory capabilities for diagnosis and treatment of HIV/AIDS in resource-limited settings

| Diagnosis and monitoring laboratory tests | | Primary care level | District level | Regional/central level |
|---|--|------------------------|----------------|------------------------|
| HIV antibody testing | | Yes ^a | Yes | Yes |
| Haemoglobin | | Desirable ^b | Yes | Yes |
| Pregnancy testing | | Desirable ^c | Yes | Yes |
| Basic microscopy for TB and malaria (sputum smear for TB and blood film for malaria diagnosis) | | Desirable ^d | Yes | Yes |
| FBC and differential | | No | Yes | Yes |
| CD4+ cell count | | No | Yes | Yes |
| ALT | | No | Yes | Yes |
| Diagnostic tests for treatable HIV coinfections and major AIDS-related opportunistic diseases | Full cerebrum spinal fluid (CSF) microscopy (including India ink for cryptococcal meningitis), syphilis and other STI diagnostic tests. | No | Yes | Yes |
| | Diagnostic tests for other major treatable HIV coinfections and AIDS-related opportunistic diseases (hepatitis B, hepatitis C serology, bacterial microbiology and cultures and diagnostic tests and procedures for <i>Cryptococcus</i> , toxoplasmosis and other major OIs) | No | Desirable | Yes |
| Full chemistry (including but not restricted to liver enzymes, renal function, glucose, lipids, amylase, lipase and serum electrolytes) | | No | No | Yes |
| HIV VL measurement | | No | No | Desirable ^e |

a Rapid tests recommended at primary care level and conventional methodologies can be used at district and regional/central levels. For details see specific WHO recommendations.

b Should be available if ZDV is being considered for use.

c Should be available if efavirenz is being considered for use.

d Referral if microscopy not available.

e VL test is currently not recommended for decision-making on initiation or regular monitoring of ART response in resource-limited settings. Use should be considered primarily for definitive diagnosis of HIV infection in children under 18 months of age who are vertically exposed to HIV during pregnancy and to assist in decision-making in more complex cases

Table 2

Frequency of laboratory tests needed for treatment of HIV/AIDS in resource-limited settings

| Diagnosis and monitoring laboratory tests | Baseline | Monthly during first 3 months (weeks 4, 8 and 12) | Every 6 months | As required (i.e. symptom-directed or if clinically indicated) |
|---|--|---|----------------|--|
| HIV antibody testing | X | | | |
| Haemoglobin ^a | X | X | | X |
| Pregnancy testing | | | | X |
| Basic microscopy for TB and malaria (sputum smear test for TB and thick blood drop smear test for malaria diagnosis) | | | | X |
| FBC and differential | X | X | | X |
| CD4+ cell count | X | | X | |
| ALT ^b | | | | X |
| Diagnostic tests for treatable HIV coinfections and major AIDS-related opportunistic diseases | Full CSF microscopy (including India ink for cryptococcal meningitis), diagnostic tests for syphilis and other sexually transmitted diseases. Diagnostic tests for other major treatable HIV coinfections and AIDS-related opportunistic diseases (hepatitis B and C serology, bacterial microbiology and cultures and diagnostic tests and procedures for <i>Cryptococcus</i> , toxoplasmosis and other major OIs) | | | X |
| Full chemistry (including but not restricted to liver enzymes, renal function, glucose, lipids, amylase, serum electrolytes, etc.) ^c | | | | X |
| HIV VL measurement ^d | | | | X |

- a Haemoglobin monitoring during the first weeks of treatment has been recommended by some experts if ZDV is used. However, other experts suggest that the haemoglobin measurement can be monitored in a symptom-directed approach, particularly for ZDV-free regimens.
- b The predictive value of pre-emptive liver enzyme monitoring is considered very low by some specialists and they recommend ALT monitoring in a symptom-directed approach in any situation. However, regular monitoring during the beginning of treatment (in monthly schedule during the first 3 months) and symptom-directed monitoring thereafter has been considered by some experts for patients using NVP-based regimens with high baseline CD4 cell count, particularly women with CD4 cells > 250/mm³ and patients with hepatitis B or C coinfection, particularly with evidence of active hepatic disease.
- c The regular monitoring (baseline and thereafter every 6 months) of full chemistry tests, particularly lipids, liver tests, renal function and glucose, has been recommended for patients using second-line drugs (see Table 3).
- d VL measurement is not currently recommended for decision-making on initiation or regular monitoring of ART response. Its use should be considered primarily for definitive diagnosis of HIV infection in children under 18 months of age who are vertically exposed to HIV during pregnancy or in special circumstances, as a complementary evaluation of more complex cases.

Group work and recommendations

Table 3

Basic recommendations for laboratory test monitoring of major first-line and second-line ARV drugs used in resource-limited settings

| ARV drug | Major potential ARV-associated adverse effects that can be monitored using laboratory tests | Laboratory test required | Monitoring frequency |
|---|---|--|--|
| Abacavir | None | None | None |
| Didanosine | Pancreatitis | Pancreatic function (amylase, lipase) | Symptom-directed |
| Efavirenz | Teratogenesis (first trimester) | Pregnancy test | Before initiation of efavirenz-based regimens in pregnant women during the first trimester |
| Indinavir/r | Renal toxicity (kidney stones), dyslipidaemia, hyperglycaemia, diabetes, hepatitis | Glucose, lipids, renal function (creatinine or urea, urinalyses), liver enzymes. | Baseline and thereafter every 6 months ^b |
| Lamivudine | None | None | None |
| Lopinavir/r | Dyslipidaemia, hyperglycaemia, diabetes, hepatitis | Glucose, lipids, liver enzymes | Baseline and thereafter every 6 months ^a |
| Nelfinavir | Dyslipidaemia, hyperglycaemia, diabetes, hepatitis | Glucose, lipids, liver enzymes | Baseline and thereafter every 6 months ^a |
| Nevirapine | Hepatotoxicity (drug-induced hepatitis) | ALT | Symptom-directed ^b |
| Saquinavir/r | Dyslipidaemia, hyperglycaemia, diabetes, hepatitis | Glucose, lipids, liver enzymes | Baseline and thereafter every 6 months ^a |
| Stavudine | None | None | None |
| Tenofovir | Renal toxicity (renal tubular injury) | Renal function (creatinine or urea, urinalyses) | Baseline and thereafter every 6 months ^c |
| Zidovudine (also known as AZT or azidothymidine) | Haematological toxicity (anaemia). | Hb | Baseline and 4, 8 and 12 weeks; thereafter symptom-directed |

a Liver function tests at this frequency have been considered by some experts for patients with hepatitis B or C coinfection, particularly with evidence of active hepatic disease. However, the predictive value of monitoring liver enzymes has been considered very low by some specialists and they recommend the use of liver test monitoring in a symptom-directed approach in any situation.

b The predictive value of pre-emptive liver enzyme monitoring is considered very low by some specialists and they recommend ALT monitoring in a symptom-directed approach in any situation. However, regular monitoring during the beginning of ARV treatment (in a monthly schedule during the first 3 months) and symptom-directed thereafter has been considered by some experts for patients using nevirapine-based regimens and with high baseline CD4 cell counts, particularly women with CD4 cells > 250/mm³ and in patients with hepatitis B or C coinfection, especially where there is evidence of active hepatic disease.

c Monitoring renal function at this frequency is particularly recommended in patients at risk for, or with a history of, renal disease, or where potentially nephrotoxic drugs are used concomitantly. In other situations the experts recommend a symptom-directed approach.

Group work 2. Review of current laboratory requirements for antiretroviral therapy roll-out: laboratory experts

The points for discussion were as follows.

What technologies are available? What needs to be evaluated? What is in the pipeline?

- ▶ Selection of appropriate technologies for different health care levels.
 - CD4; VL; basic laboratory tests, others
 - > What is available?
 - > What are the parameters?
(See draft table for CD4.)
 - > What are acceptable quality standards?
- ▶ Review minimum laboratory requirements and amend where necessary.
- ▶ Need for CD4 counts at primary health care level – is this realistic? Will depend on tests currently in development
- ▶ More guidance needed on sample collection/ storage/transport, stability of samples for sample referrals to higher-level laboratories
- ▶ QA/QC issues
- ▶ Service and maintenance

The following recommendations were made.

- ▶ There should be a clear understanding of the targeted population for CD4+ T-cell monitoring, e.g. adults versus children.
- ▶ The performance and quality of technologies should be assessed and validated by means of multisite evaluation studies, with independent data analysis, e.g. through WHO.
- ▶ The selection of appropriate technologies should be based on scientific evidence, the estimated current and future workload (number of specimens to be processed daily), the volume throughput, the physical laboratory infrastructure, the capacity of laboratory staff and the availability of technical maintenance support.
- ▶ Demographic forecasts should be obtained with reference to rural versus urban numbers, the prevalence and incidence of HIV, volume catch areas and national coverage.

- ▶ The option of centralized testing should be considered with reference to sample collection and storage/transport, as should QA/QC issues, e.g. the use of samples from the previous day.
- ▶ CD4 determinations were not required at the primary health care level. Either patients or specimens could be referred to district or provincial laboratories.
- ▶ Service and maintenance contracts should be obtained when sophisticated equipment was being purchased. WHO should advocate to manufacturers that capacity be developed for better service from local distributors at the country or regional level.
- ▶ WHO should provide training and develop training materials, including generic SOPs, for laboratory tests supporting the roll-out of ART.
- ▶ WHO and its partners should continue to negotiate reduced prices for HIV/AIDS-related technologies.

Group work and recommendations

Group work 3. Scale-up: What is needed?

The points for discussion were as follows.

- ▶ National short-term and long-term plan
 - > National coverage (How many units of each type of equipment and where are they needed?)
 - > Current and future estimation of needs for each of the laboratory technologies
 - > Degree of standardization at national level
 - > Organization and management of laboratories / testing sites (including supervision)
 - > Allowance for flexibility, introduction of future technologies
 - > Sustainability
- ▶ Centralization versus decentralization (logistics)
- ▶ Infrastructure requirements and technology requirements
 - > Scale-up of ART with scale-up of laboratory support
 - > Catering for all types of patients (adults and children)
- ▶ Procurement and supply management issues
- ▶ Local capacity for maintenance of technologies, good service delivery (knowledgeable local distributors)
- ▶ Training on technologies, appropriate use of technologies, SOPs
- ▶ QA

The following recommendations were made.

- ▶ Each ministry of health should establish a national plan of laboratory support for ART roll-out with a specific budget component. Input from both clinical and laboratory experts, people living with HIV/AIDS, and relevant programmes, e.g. TB, NGOs, should be incorporated. Communication and collaboration between all parties should be fostered.
- ▶ In order to ensure the sustainability of programmes, budget allocations should be realistic, covering not only the purchase of equipment and reagent costs but also transport, data entry, training, maintenance and QA. Long-term political

commitment is vital for the continuity of supplies, staff retention and annual strategic planning reviews.

- ▶ Health ministries should estimate how many patients require treatment within the budget allocation at each level of the health care setting and should forecast the volume of testing accordingly.
- ▶ Each health ministry should establish both a clinical and a laboratory referral network. Minimum requirements for human resources and infrastructure for both networks should be defined. The impact on the other services should not be compromised. An integrated data management system covering clinical and laboratory data, and linkages between centralized and decentralized laboratories, should be set up.
- ▶ Each health ministry should develop a short-term (1–2-year) and longer-term (5-year) forecast plan in respect of equipment needs, covering the range of tests, the anticipated volumes and the available suppliers. When contracts are being negotiated it is necessary to consider training requirements, maintenance, QA and technological developments. If new, more appropriate technology is likely to become available shortly it may be wise to lease rather than buy current technology.
- ▶ Each health ministry should, as far as possible, standardize the technologies used (CD4, VL, haematology, clinical chemistry, HIV serology, OI, STI, TB, etc) at each health care level. This approach should cover training, SOPs, QA programmes and supervision.
- ▶ WHO should develop generic plans for each of the elements/components of a national laboratory and ARV support plan.
- ▶ WHO should develop generic SOPs for the main technologies and maintain and expand QA activities, including guidelines and training on GLP.
- ▶ WHO should rapidly disseminate new information on relevant technologies.
- ▶ WHO should build capacity for the local evaluation of technologies.
- ▶ Procurement and supply management at the country level should be strengthened with the help of WHO and its partners.

Group work 4. Innovative and operational research

The discussion covered the following points.

What specifications, operational characteristics and costs for technologies appropriate in resource-poor settings are required?

- > Simplified techniques for ease of use (less specialized training/support; suitable for children)
- > Electricity
- > Temperature and humidity
- > Cost of equipment and reagents
- > Dried fluid spots
- > QC
- ▀ What technologies are currently under development and when are they expected to become available on the commercial market?
- ▀ Current technologies; develop and assess test procedures for paediatric use
- ▀ Are all current technologies suitable for monitoring?
 - > CD4 manual methods?
- ▀ What are the optimal time intervals for determining:
 - > CD4 levels?
 - > Do TLCs equal CD4 counts?
 - > Toxicity tests?
 - > Early diagnosis infants with RNA tests?

The following recommendations were made.

- ▀ In five years it should be possible to diagnose HIV infection and monitor ARV in infants, children and adults at the primary health care level.
- ▀ In five years it should be possible, at the district level, to diagnose HIV infection, to monitor therapy with CD4 counts, to diagnose and treat common OIs (TB, toxoplasmosis, cryptococcal diseases), identify toxicity and flag patients (infants, children and adults) in whom therapy is failing, ideally with a multiplex instrument. WHO should advocate the development of such instruments

Currently, there were alternative options for fulfilling these tasks. Once validated, the PointCare instrument would come close to meeting the CD4 monitoring needs. However, QA/QC would have to be developed.

- ▀ WHO should facilitate the development of innovative diagnostics that are simple, rapid, robust and inexpensive point-of-care assays for absolute and percentage CD4 counts and for the diagnosis of infants aged up to 2–3 months.
- ▀ The dried blood or fluid spot referral system approach for DNA PCR and VL for early diagnosis of infants should be validated under field conditions.
- ▀ Independent comparative multisite evaluations of new CD4 technologies, including Cyflow Counter, Cyflow Green, EasyCD4, PointCare, LabNow and Semibio versus the gold standard methods (flow cytometry and FACSCount) should be conducted once the technologies are commercially available. The tolerance for accuracy divergence should be established on clinical needs, with reference to what is acceptable (1%, 5%, 10% or 20% away from the gold standard if other specifications are met).
- ▀ Independent multisite comparative evaluation of VL assays, e.g. ExaVir and Retina Rainbow, should be conducted once commercially available. (WHO had a pilot comparative evaluation at one site.)
- ▀ The performance of all assays should be subject to appropriate quality measures, including built-in QC as well as external quality assessment.
- ▀ Strong linkages should be established between clinical sites and laboratory sites, and databases correlating clinical and laboratory outcomes should be set up. Harmonization was vital.
- ▀ WHO should conduct a clinical study to define treatment failure and should assess the impact of early versus late switching to second-line regimens.
- ▀ WHO should develop a standardized adherence questionnaire and investigate its value for making treatment decisions.
- ▀ More precise guidance was needed to determine the best switch options on the basis of clinical findings.

Group work and recommendations

- ▶ The utility of CD4 and VL testing in the clinical management of patients on ART living in resource-limited settings should be investigated in other trials like DART.
- ▶ WHO should validate the utility of manual CD4 methods as tools for initiating and monitoring ART and making appropriate clinical decisions concerning patients on ART in resource-limited settings.
- ▶ WHO should validate the utility of total lymphocyte counts as tools for initiating and monitoring ART and making appropriate clinical decisions for patients on ART.
- ▶ The utility of toxicity tests (ALT) for clinical management should be investigated.
- ▶ The influence of viral clades and coinfections (TB, malaria, etc.) on the effectiveness of current assays and monitoring protocols should be studied.
- ▶ Reference values for CD4 for adults and infants ≤in countries most affected by the epidemic should be collated.
- ▶ WHO should promote even more strongly the combination of two or three rapid HIV tests to confirm HIV seropositivity.
- ▶ WHO should conduct a survey of CD4 technologies in order to compare them in respect of cost per patient-result, daily throughput, days lost to equipment failure, staffing levels and training requirements.
- ▶ WHO should promote the development and validate the reliability of simple bedside VL tests.
- ▶ WHO should conduct studies to determine the VL level appropriate to triggering a clinical decision to switch therapy. (Were there other simple inexpensive laboratory tests? What was the role of hepatitis B virus and hepatitis C virus screening in relation to treatment regimens with nevirapine and lamivudine)
- ▶ WHO should conduct studies on the value of VL as an adherence tool.
- ▶ WHO should develop and validate a standardized patient questionnaire for the assessment of adherence and should compare both adherence tools.
- ▶ WHO should support the development and validate the reliability of new technologies that detect latent TB infection and can diagnose smear-negative active TB infections in under 24 hours.
- ▶ WHO should assess the performance of currently available technologies for the early detection of HIV infection in children and should provide guidance on HIV diagnosis in children. (What were the technologies and what were the time intervals for specific available technologies?)
- ▶ WHO should support the development of new simple technologies for detecting ARV resistance.

There was no agreement concerning further studies on manual CD4 methods. WHO was potentially interested in validating a reverse external quality assessment approach for manual methods. However, if manual methods were becoming obsolete this might no longer be a priority.

Closing Remarks

Dr Gilks thanked the participants for their commitment and three days of hard work. Bringing the clinicians and laboratory experts together at the global level had been very fruitful and it was desirable for similar meetings to be held at the regional and national levels.

Annex 1. Programme of work

Monday 13 December 2005

| | | |
|---------------|---|-------------------------------------|
| 08:30 – 09:00 | Registration | |
| 09:00 – 09:30 | Opening address | Dr Jim Yong Kim, Director, HIV/AIDS |
| 09:30 – 10:00 | Objectives of the meeting and review of current WHO recommendations | Dr Charles Gilks, Coordinator, TPS |
| 10:00 – 10:15 | Review of HIV/AIDS diagnostic support activities | Dr Gaby Vercauteren |
| 10:15 – 10:30 | Discussion | |
| 10:30 – 10:45 | Tea/coffee | |
| 10:45 – 11:00 | Scale-up experience in country (Kenya) | Dr Sylvia Ojoo |
| 11:00 – 11:15 | Scale-up experience in country (Senegal) | Dr Salif Sow |
| 11:15 – 11:30 | Scale-up experience in country (Thailand) | Dr Anupong Chitwarakorn |
| 11:30 – 11:45 | Scale-up experience in country (MSF) | Dr Alexandra Calmy |
| 11:45 – 12:00 | Scale-up experience in country (Brazil) | Dr Mauro Schechter |
| 12:00 – 13:00 | Discussion | |
| 13:00 – 14:00 | Lunch | |
| 14:00 – 14:20 | Clinical issues: monitoring adults | Dr Robert Colebunders |
| 14:20 – 14:40 | Clinical issues: monitoring children | Dr Gabriel Anabwani |
| 14:40 – 15:00 | Resistance | Dr Don Sutherland |
| 15:00 – 15:20 | Technology for resistance monitoring | Dr Martine Peeters |
| 15:20 – 15:40 | CD4 technologies | Dr Frank Mandy |
| 15:40 – 16:00 | Tea/coffee | |
| 16:00 – 16:10 | CD4 technologies (Partec) | Dr Luc Kestens |
| 16.10 – 16:20 | CD4 determinations for paediatrics | Dr Frank Denny |
| 16:20 – 16:40 | VL technologies and incidence assay | Dr Elizabeth Dax Taylor |
| 16:40 – 17:00 | ARV toxicity monitoring (haematology / clinical chemistry) | Dr Wendy Stevens |
| 17:00 – 17:20 | TB/HIV laboratory issues | Dr Pierre-Yves Norval |
| 17:20 – 18:00 | Discussion | |

Tuesday 14 December 2005

| | | |
|---------------|--|---------------------|
| 09:00 – 09:15 | Review of day 1 | Rapporteurs |
| | Procurement issues | |
| 09:15 – 09:30 | WHO prequalification process | Dr Olivier Gross |
| 09:30 – 09:40 | WHO selection of appropriate diagnostic technology | Ms Anita Sands |
| 09:40 – 10:00 | WHO procurement – CPS | Ms Françoise Mas |
| 10:00 – 10:15 | UNICEF | Dr Ludo Scheerlinck |
| 10:15 – 10:30 | AMDS | Dr Jos Perriëns |
| 10:30 – 10:45 | Discussion | |
| 10:45 – 11:00 | Tea/coffee | |
| 11:00 – 13:00 | Working groups Review of clinical recommendations: Groups A and B Review of laboratory recommendations: Groups C and D | |
| 13:00 – 14:00 | Lunch | |
| 14:00 – 15:00 | Continuation of working groups | |
| 15:00 – 15:10 | Presentations: Clinical Group A | |
| 15:10 – 15:20 | Presentations: Clinical Group B | |
| 15:20 – 15:45 | Discussion | |
| 15:45 – 16:00 | Tea/coffee | |
| 16:00 – 16:10 | Presentation: Laboratory Group C | |
| 16:10 – 16:20 | Presentation: Laboratory Group D | |
| 16:20 – 17:00 | Discussion | |

Annex 1. Programme of work

Wednesday 15 December 2005

| | | |
|---------------|--|-------------|
| 09:00 – 09:30 | Review of day 2 | Rapporteurs |
| 09:30 – 10:45 | Group work. Scale-up: what is needed? Clinical Group A and Laboratory Group C Group work: Innovative and operational research: Clinical Group B and Laboratory Group D | |
| 10:45 – 11:00 | Tea/coffee | |
| 11:00 – 11:50 | Group work continued | |
| 11:50 – 12:00 | Presentation of group work: Scale-up, clinical | |
| 12:00 – 12:30 | Discussion | |
| 12:30 – 12:40 | Presentation of group work: Scale-up, laboratory | |
| 12:40 – 13:00 | Discussion | |
| 13:00 – 14:00 | Lunch | |
| 14:00 – 14:10 | Presentation of group work: Research agenda, clinical | |
| 14:10 – 14:30 | Discussion | |
| 14:30 – 14:40 | Presentation of group work: Research agenda, laboratory | |
| 14:40 – 15:00 | Discussion | |
| 15:00 – 15:15 | Updated recommendations: Plenary | |
| 15:15 – 15:45 | Discussion | |
| 15:45 – 16:00 | Tea/coffee | |
| 16:00 – 16:30 | Recommendations and closure | |

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Annex 3. Summary of CD4+ T-cell enumeration technologies: flow cytometry

| Parameter | Double platform ^a | Single platform | |
|-------------------------------------|--|---|--|
| | | Volumetric ^b | Bead-based ^c |
| Instruments, manufacturers | Flow cytometer Partec GmbH (Münster, Germany) ^d Becton Dickinson (California, USA) Coulter Corporation (Florida, USA) | Flow cytometer Partec GmbH (Münster, Germany) ^d Guava Technologies (California, USA) | Flow cytometer Becton Dickinson (California, USA) Coulter Corporation (Florida, USA) |
| Cost of instrument (US\$) | 20 000–95 000 | 20 000–70 000 | 20 000–95 000 |
| Cost of reagents/test (US\$) | 3–11 | 2–10 | 8–25 |
| Specimen | Whole blood | Whole blood | Whole blood |
| Results | Absolute CD4 count Absolute CD8 count CD4 % and CD8 % among lymphocytes CD4/CD8 ratio B and NK cells are possible ^e | Absolute CD4 count Absolute CD8 count CD4 % and CD8 % among lymphocytes CD4/CD8 ratio B and NK cells are possible | Absolute CD4 count Absolute CD8 count CD4 % and CD8 % among lymphocytes CD4/CD8 ratio B and NK cells are possible |
| Throughput (samples/day) | Up to 200 | Up to 50 | Up to 200 |
| Advantages | Accurate pipetting less crucial One tube assay possible without QC problems EQA available | No need for extra beads or haematology analyser Protocols for aged samples available EQA available | No need for haematology analyser Protocols for aged samples available EQA available |
| Disadvantages | Requires the use of a haematology analyser More prone to clerical errors Fresh samples needed in order to obtain absolute counts | Requires accurate pipetting technique Internal QC for pipetting requires two tubes assay Instruments not yet proven an independent multicentre study | Requires accurate pipetting technique Internal QC for pipetting requires two tubes assay Beads are expensive and require careful handling |

- a Any flow cytometer from any of the three manufacturers can operate with this method to provide absolute counts. The results of flow cytometry are combined with those from haematology in order to calculate absolute counts.
- b Volumetric instruments have the inherent hardware property of measuring the volume of the sample, providing direct absolute counts without the use of haematology analysers or beads.
- c Any flow cytometer from any of the three manufacturers can operate with this method to provide absolute counts.
- d Instruments from this manufacturer, including the CyFlow, remain to be validated as volumetric absolute CD4 T-cell counters by independent investigators in multicentre studies.
- e B and NK cells are subsets of lymphocytes.

Annex 4. Summary of CD4+ T-cell enumeration technologies: dedicated and manual assays

| | Dedicated technology | | Manual assays | |
|--|---|---|---|---|
| | FACSCount | CyFlow Counter | Cytospheres | Dynabeads |
| Manufacturer | Becton Dickinson (California, USA) | Partec GmbH (Münster, Germany) | Coulter Corporation (Florida, USA) | Dynal AS (Oslo, Norway) |
| Instrumentation | Dedicated CD4 counter | Dedicated CD4 counter | Haemocytometer Light microscope | Magnet Haemocytometer Light or fluorescence microscope |
| Assay principle | Flow cytometry | Flow cytometry | Direct observation of bead-rosetted cells | Direct observation of immunocaptured cells |
| Detection system | Fluorochrome-labelled anti-CD3, CD4 and CD8 (Mab) | Fluorochrome-labelled anti-CD4 Mab | Latex beads conjugated to anti-CD4 Mab | Magnetic beads conjugated to anti-CD4 and CD8 Mab |
| Specimen | Whole blood | Whole blood | Whole blood | Whole blood |
| Results | Absolute CD4 and CD8 counts CD4/CD8 ratio CD4 % and CD8 % among T-cells | Absolute CD4 count | Absolute CD4 count | Absolute CD4 count Absolute CD8 count CD4/CD8 ratio |
| Correlation with flow cytometry^a (r value) | 0.93–0.98 (several international studies) | | 0.67–0.93 (several international studies) | 0.94 and 0.96 (several international studies) |
| Cost of instrument (US\$) | 28 000 | 20 000 | 2000 | 2000–10 000 ^b |
| Cost of reagents/test (US\$)^c | 6–20 | 2 | 4–8 | 3–5 |
| Advantages | Automated, fewer steps, less human error, low biohazard risk Absolute CD4 and CD8 counts Quick results EQA available | Reagents available at low cost Quick results EQA available | Simple and rapid | Simple and rapid Absolute CD4 and CD8 counts |
| Disadvantages | Expensive reagents 12 samples processed at a time CD4 % among lymphocytes not reported | CD4 % among lymphocytes not reported Instrument not proven in an independent multicentre study | 10 samples processed at a time Subjectivity in visual counting CD4 % among lymphocytes or CD8 counts not reported No EQA available | 6 samples processed at a time Subjectivity in visual counting CD4 % among counts not reported No EQA available |

a The analysis of correlation using linear regression is not appropriate for comparison of methods. Instead, analysis of agreement should be performed. Unfortunately, none of the published studies has used this analysis to compare these methods with flow cytometry. The r values are therefore reported here.

b Depending on whether a light microscope or a fluorescence microscope is used.

c Equipment cost may vary and reagent cost may decrease substantially in the near future.

Annex 5. Summary of main characteristics of viral load technologies based on nucleic acid

| Company | Abbott | | Roche | | Bayer | | bioMérieux | | Primagen | |
|---------------------------------|---|----------------------------------|---|---|---|---|---|---|---|------------------------|
| Assay name | LcX HIV RNA Quantitative | | Amplicor HIV-1 Monitor Test | | Versant HIV-1 RNA 3.0 Assay | | NucliSens EasyQ HIV-1 | | Retina Rainbow | |
| Type of assay | RT-PCR | | RT-PCR | | bDNA | | RT-NASBA | | RT-NASBA | |
| Dynamic range (copies/ml) | 50–1 000 000 | | 50–750 000 | | 75–500 000 | | 50–3 000 000 | | 500–50 000 000 | |
| Specimen type | Plasma | | Plasma, dried blood spots | | Plasma | | Plasma, serum, dried blood spots | | Plasma, serum, whole blood, dried blood spots | |
| Specimen volume | 200–1 000 ml | | 100–500 ml | | 1000–2000 ml | | 10–2000 ml | | 200 ml | |
| Area of HIV genome amplified | Pol | | Gag | | Pol | | Gag | | LTR | |
| HIV-1 subtypes amplified | Group M (subtypes A to G) and Group O | | All, plus some HIV-2 | | Group M (subtypes A to G) | | All | | All | |
| Time for result | 5 hours | | 6–7 hours | | 22 hours | | 2.5–3 hours | | 1.5 hours | |
| Cost/test ^a | \$20–70 | | \$28–90 | | \$125 | | \$38–76 | | \$17–23 | |
| Number of samples/run | 21 (+3 controls) | | 9–48 | | 12–168 | | 8–48 | | 96 | |
| Equipment required ^b | Vacuum pump Centrifuge (x 2) Heat block | LCx analyser Thermal cycliser | COBAS Ampliprep Dead-air box Computer/printer | Safety hood Heat block (x 2) Centrifuge (x 2) | Bayer System 340 (bDNA analyser, data management and computer system) | Centrifuge Heatblock Waterbath Vacuum system | Nucli Sens miniMAG system/ Nuclisens east MAG system | Nucli Sens EasyQ analyser Strip centrifuge | Retin Alyser Heatblock | Computer Centrifuge |
| Equipment cost (US\$) | 8 500 + LCx analyser 25 000 | | 10 000 + COBAS Ampliprep 30 000 | | 10 000 + Bayer System Analyser | | | | 23 000 | |

a Prices vary considerably with quantities and special negotiations.

b All assays require pipettes and vortex mixers; refrigerator required for all but Primagen.

Annex 6. Summary of main characteristics of viral load technologies not based on nucleic acid

| Company | Cavidi | Perkin Elmer |
|---|---|--|
| Assay name | ExaVir [®] Load Quantitative HIV-RT Load Kit | HIV-1 p24 Ultra ELISA ELAST ELISA amplification system |
| Type of assay | Enzyme immunoassay for quantitation of RT activity | Enzyme immunoassay for quantitation of p24 antigen |
| Dynamic range | 750 to over 50 000 copies/ml | pg/ml 400 copies/ml |
| Specimen type | Plasma | Plasma, serum or cell culture supernatant |
| Specimen volume | 1000 ml | 100 ml |
| Area of HIV genome selected for amplification | RT activity | p24 antigen |
| HIV-1 subtypes amplified | All plus HIV-2 | HIV-1 |
| Time for result | 24 hours | 6 hours |
| Cost/test | \$13 –115 | \$10 |
| Number of samples/run | 30 | 96 |
| Equipment required ^a | Incubator (33 °C), freezer, ELISA reader, computer | Incubator, ELISA reader, refrigerator |
| Equipment cost (US\$) | 9000–10 000 (start-up pack includes other necessary equipment and three kits) | 7000–9000 |

a Both assays require pipettes and vortex mixers



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