

WHO/ASD/98.1
UNAIDS/98.7
Distr: General

Guidance Modules on Antiretroviral Treatments

Module 5

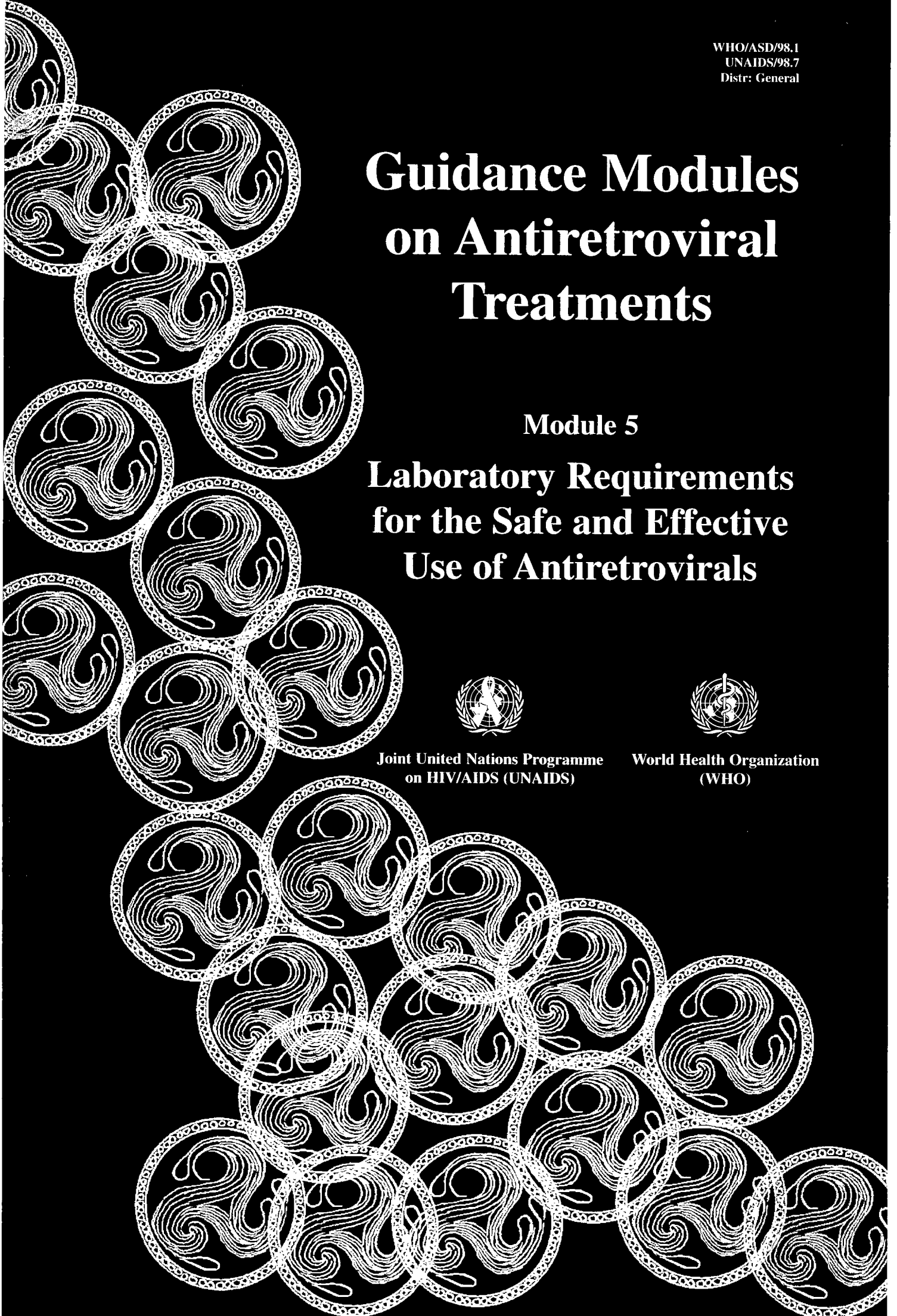
Laboratory Requirements for the Safe and Effective Use of Antiretrovirals



Joint United Nations Programme
on HIV/AIDS (UNAIDS)



World Health Organization
(WHO)



Acknowledgements

The World Health Organization and UNAIDS would like to thank Professor Gunnel Biberfeld, Swedish Institute for Infectious Disease Control, Stockholm, Sweden, for preparing the text of this module;

Dr Gaby Vercauteren, Blood Safety Unit, WHO, Dr Frank De Wolf, University of Amsterdam, the Netherlands and Professor Nkandu Luo, University Teaching Hospital, Lusaka, Zambia, for their most thoughtful reviews of the draft text; and

Dr Rachel Baggaley, Dr Susan Fernyak, Ms Alison Martin Katz, Ms Marguerite Nguyen and Dr Eric van Praag, Office of HIV/AIDS and Sexually Transmitted Diseases, WHO, and Dr Joseph Perriens, Policy, Strategy and Research, UNAIDS, for coordinating the project.

©World Health Organization and Joint United Nations Programme on HIV/AIDS, 1998

This document is not a formal publication of the World Health Organization (WHO) and Joint United Nations Programme on HIV/AIDS (UNAIDS), and all rights are reserved by them. The document may, however, be freely reviewed, abstracted, reproduced, or translated in part, but not for sale or for use in conjunction with commercial purposes. It may also be reproduced in full by non-commercial entities for information or for educational purposes with prior permission from WHO and UNAIDS.

For authorization to translate or reproduce the work in full, and for any use by commercial entities, applications and enquiries should be addressed to the Office of HIV/AIDS and Sexually Transmitted Diseases, World Health Organization, 1211 Geneva 27, Switzerland, which will be glad to provide the latest information on any changes made to the text, plans for new editions, and the reprints and translations that are already available.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by either WHO or UNAIDS in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

The views expressed in these documents by named authors are solely the responsibility of those authors.

Module 5

Laboratory Requirements for the Safe and Effective Use of Antiretrovirals

Introduction

The introduction of combination antiretroviral treatment (ARV) for HIV infection has dramatically changed the clinical perspective for people living with HIV/AIDS, for health care professionals responsible for their care and for policy makers. This module reviews the laboratory requirements for safe and effective use of ARVs including:

- Diagnosis of HIV infection through the detection of antibodies or viral genome by polymerase chain reaction (PCR)
- Monitoring of adverse reactions to ARV drugs
- Diagnosis of opportunistic infections
- CD4+ T-cell determinations
- Viral load monitoring
- Monitoring of resistance of HIV

The importance of reliable and accurate laboratory support for ARV treatment is stressed and basic information provided on how to achieve a high standard of performance. Well trained staff is a prerequisite for a high standard of performance. Basic training and regular refresher training should be routine in the laboratories set up for ARV services. Cost estimates provided are based on commercial prices given by the manufacturers. Costs may vary between geographical areas and are often negotiable. Labour costs have not been included.

HIV testing

The standard procedure for laboratory diagnosis of infection with the human immunodeficiency virus (HIV) usually includes screening for virus specific antibodies with an enzyme-linked immunosorbent assay (ELISA) or a rapid simple test, followed by confirmatory testing of screened positive samples¹.

The most widely used screening tests are ELISAs which comprise a number of variants based on different principles including indirect, competitive, sandwich and capture assays, all of which may detect HIV-1 as well as HIV-2 antibodies. Most of the first generation ELISA tests were based on viral lysate antigens. Later variants utilise recombinant proteins and/or synthetic peptides. The latter are generally more sensitive and specific as there are fewer contaminating products such as cellular components in the viral lysate. Through a WHO initiative, extensive evaluations of HIV antibody assays have been performed.

The antibody screening assays, though very sensitive, specific and reproducible, are not completely free from false reactions. The most common testing strategy for detection of HIV

¹ Joint United Nations Programme on HIV/AIDS (UNAIDS)/WHO. Revised recommendations for the selection and use of HIV antibody tests. Weekly Epidemiological Record 1997;72:81-88.

antibodies is therefore to perform a second more specific supplementary (confirmatory) assay on sera repeatedly reactive on a screening assay. Western blot (WB) and/or line immunoassays (LIA, RIBA) are tests used specifically for serological confirmation.

WB tests are expensive, time consuming and have technical disadvantages, such as lack of standardisation in performance and interpretation, and indeterminate reactions. Therefore, alternative strategies for confirmation of HIV antibody positive samples that can replace conventional WB testing have been evaluated. WHO has issued guidelines on testing strategies according to the purpose of testing. The general principle is to use a combination of cheaper and less sophisticated tests, e.g. two or three ELISAs or simple/rapid tests, in such a way that sensitivity and specificity will be comparable to the more commonly used strategies including confirmation by WB. Ideally, an alternative strategy for confirmation of HIV infection should consist of a combination of assays of different test principles and different antigen preparations. It is also possible to design an alternative strategy for confirmation to include discrimination between HIV-1 and HIV-2. Furthermore, the complete test procedure (from receiving the sample to sending out the final result), using the alternative confirmatory strategy, saves time. Positive samples may be confirmed and results submitted to the referring doctors the same day as they have been screened.

Apart from standard laboratory equipment, ELISA testing requires a spectrophotometer at a cost of around US\$ 10,000. Simple/rapid test kits are often supplied with all the reagents needed while basic utensils such as serum tubes, pipettes and a simple centrifuge for separation of serum have to be provided by the laboratory. For storage of test kits a refrigerator is required. Serum can be kept in a refrigerator for some time but for longer storage they should be frozen to at least -20°C . ELISA tests for HIV can be purchased from around US\$1 per test, while simple/rapid tests are usually more expensive (from around US\$3). Western blot tests cost US\$ 20 - 30. Many HIV antibody tests can be obtained through WHO bulk purchase at a reduced rate.

Apart from HIV antibody measurements, detection of virus genome by nucleic acid amplification techniques (NAT), such as the polymerase chain reaction (PCR), has a place in certain cases for confirmation of HIV infection. NAT are particularly useful for further characterisation of virus strains, such as subtyping, monitoring of resistance to ARV drugs, and measurement of viral load. PCR is also the main mode of diagnosis of HIV in infants. In settings with limited laboratory facilities, modified heat-denatured antigen detection may be used as an alternative to PCR for the early diagnosis of HIV infection in infants. While antigen detection is mainly based on the same principles as antibody testing, NAT are sensitive and complicated methods requiring special laboratory facilities, instruments and skills, and careful quality assurance. For further information on PCR and related NAT, see the sections on viral load monitoring and monitoring resistance of HIV.

Finally, it should be noted that when HIV infection has been detected for the first time in a person, the diagnosis should always be reconfirmed on a second blood sample, in order to exclude laboratory and administrative mistakes.

In conclusion, **laboratory diagnosis of HIV infection** is usually done initially by the detection of HIV specific antibodies in serum or plasma. Antibody detection can be done with simple/rapid tests which require a minimum of laboratory equipment and material. Testing

strategies based on simple/rapid assays are most suitable for small numbers of specimens. For larger numbers of specimens it is more practical and economic to use ELISAs. These assays require larger investments in equipment and a regular supply of electricity and water. **Detection of the virus itself**, through antigen detection or NAT is usually used for specific purposes, e.g. during early stages of infection when antibody levels may be low, for diagnosis in infants born to mothers with HIV, for subtyping of virus strains, or for determination of virus load.

Monitoring of patients for adverse reactions to ARVs

Like most potent medical drugs, ARVs may cause adverse reactions which have to be weighed against the potential benefits. Adverse reactions vary from one drug to another and between individual patients. Some are of a general character and can be monitored clinically, e.g. skin rashes, nausea, headache and polyneuropathy. Some adverse reactions decline after time. Other adverse reactions can be monitored with laboratory tests. The major ARV drugs, their most important side effects and the modes of detection and follow-up of these adverse reactions are summarised in Table 1.

Among nucleoside reverse transcriptase inhibitors (NRTIs), zidovudine may cause anaemia and neutropenia. Lamivudine has relatively few side effects while the other NRT inhibitors can occasionally cause pancreatitis. Thus, regular blood counts and analyses of amylase in serum (for symptomatic patients) are required. Among the protease inhibitors, indinavir may cause elevated bilirubin levels and clinical nephrolithiasis with pain in the flank and hematuria (reported in 3-5% of all patients). The most common adverse events reported for the other protease inhibitors are diarrhoea and other gastrointestinal disturbances. Protease inhibitors have also been reported to cause liver enzyme elevations, lipodystrophy, and diabetes mellitus in a few cases.

Timing of check-ups depends on the clinical condition of the patient and the treatment being taken. If the patient tolerates the treatment well and is in a stable clinical condition, the interval between check-ups can be extended to 3-4 months. Otherwise the intervals should be shorter according to need, as decided by the doctor in charge (see Module 4 on the safe and effective use of ARVs for further details).

In conclusion, regular clinical and laboratory monitoring of patients on ARVs is necessary. The laboratory tests to be performed depend on which ARV drugs are being used, but generally comprise blood counts including differential, as well as bilirubin and liver enzymes, amylase, urate and glucose (simple dip tests of the urine are usually sufficient). It must be emphasised that baseline laboratory analyses should be performed before ARV treatment is started.

Table 1: Antiretroviral drugs, main adverse reactions and follow-up action/tests

Drug	Adverse reactions	Follow-up action/tests
1. Nucleoside Reverse Transcriptase Inhibitors		
1.1. Zidovudine (ZDV, 3 ¹ -azido-2 ¹ , 3 ¹ - dideoxythymidine)	- initial headache and nausea (usually temporary) - anaemia, leucopenia (neutropenia) - myopathy	- clinical examination - blood count including differential - CK
1.2. Didanosine (ddI, 2 ¹ , 3 ¹ - dideoxyinosine)	- gastrointestinal disturbances - polyneuropathy (long term treatment) - pancreatitis	- clinical examination - clinical examination - amylase
1.3. Lamivudine (3TC)	(few)	
1.4. Zalcitabine (ddC, 2 ¹ , 3 ¹ - dideoxycytidine)	- polyneuropathy - ulcerative stomatitis - pancreatitis	- clinical examination - clinical examination - amylase
1.5. Stavudine (d4T, 2 ¹ , 3 ¹ - dideohydro- dideoxy-thymidine)	- peripheral polyneuropathy (common) - abnormal liver function tests (LFT) - pancreatitis (rare)	- clinical examination - liver enzymes - amylase
2. Non-nucleoside Reverse Transcriptase Inhibitors		
2.1. Nevirapine	- skin rash (common) - elevation of liver enzymes	- clinical examination - liver enzymes
2.2. Delavirdine	- skin rash (common) - abnormal LFT	- clinical examination - liver enzymes
3. Protease Inhibitors		
3.1. Indinavir	- nausea, gastrointestinal dis- turbances, headache, dry skin - elevation of bilirubin - kidney stones/flank pain - diabetes mellitus (rare) - haemolytic anaemia (rare) - liver dysfunction (rare)	- clinical examination - bilirubin - urinary dip tests (glucose, erythrocytes) - bilirubin, liver enzymes

3.2. Ritonavir	<ul style="list-style-type: none"> - nausea, gastrointestinal disturbances - paresthesias - elevation of serum levels of liver enzymes, urate, glutamyl-transpeptidase (GT), creatine-kinase (CK), triglycerides - diabetes mellitus (rare) 	<ul style="list-style-type: none"> - clinical examination - clinical examination - analysis of serum levels of: liver enzymes, urate, GT, CK, triglyceride - analysis of glucose in urine
3.3. Nelfinavir	<ul style="list-style-type: none"> -gastrointestinal disturbances (around 20 % of patients) - hyperglycaemia and lipodystrophy. 	<ul style="list-style-type: none"> - clinical examination - analysis of glucose in urine - liver enzymes

Identification and diagnosis of opportunistic infections

Opportunistic infections are significant clinical features of HIV/AIDS and their detection and treatment is of considerable importance in the care of HIV infected people. The incidence of opportunistic infections usually decreases with the introduction of ARVs. However, in cases of non-optimal compliance with ARV treatment or the development of resistance to the ARV drugs used, opportunistic infections may reappear.

A summary of the main opportunistic infections and complicating syndromes in HIV disease including the mode of diagnosis and basic equipment and reagents needed, is provided in Table 2.

Some of the opportunistic infections, like oral or oesophageal candidiasis, cytomegalovirus retinitis and cutaneous Kaposi's sarcoma (KS) may require only a clinical examination for diagnosis and follow-up (KS is often confirmed by histopathological examination of tissue specimens). Many of the parasitic infections can be diagnosed by microscopy of relevant specimens, such as stool and tissue preparations. Lymphomas will also be diagnosed and typed through histopathological examination by microscopy of tissue specimens. A pulmonary X-ray in combination with microscopy of a sputum sample is often sufficient for confirmation of pulmonary tuberculosis or pneumocystis carinii infection. Computed tomography (CT) or magnetic resonance imaging (MRI) are valuable tools in the of brain manifestations of HIV disease, such as toxoplasmosis of the brain, lymphomas and progressive multifocal leukoencephalopathy (PML). For proper confirmation of bacterial infections it is necessary to perform cultures. Tuberculosis and virus cultures require special laboratory facilities and reagents.

Thus, with a good knowledge and clinical experience, it is possible to adequately monitor many opportunistic infections with clinical examinations and basic technical facilities, such as light microscopy and chest X-ray only. Diagnostic possibilities can be extended with the addition of a bacteriological laboratory. Ideally, facilities for TB and virus cultures, CT and/or MRI scans should be available. The level of sophistication will be determined locally according to needs, available resources and existing technical facilities.

Table 2. Summary of main opportunistic infections and complicating syndromes in HIV infection, mode of diagnosis, basic equipment and reagents

Disease	Frequency ^c	Mode of diagnosis	Equipment and reagents	Priority ^b
1. Protozoan Infections				
1.1. Pneumocystis carinii	***	- clinical examination ^a - microscopy of sputum (if possible, induced sputum) - pulmonary X-ray - bronchoscopy (e.g. with BAL)	- light microscope, staining material - X-ray equipment - film and reagents for film processing - bronchoscopy equipment (for BAL also light microscope and staining material)	* * *
1.2 Toxoplasmosis	**	- clinical examination - detection of serum antibodies - CT - MRI - brain biopsy histology (exceptional)	- CT equipment - MRI equipment - (surgery), light microscope, staining material	* * (*)
1.3. Cryptosporidiasis	*	- stool microscopy	- light microscope	*
1.4. Isospora belli	*	- stool microscopy	- light microscope	*
2. Bacterial infections				
2.1. Tuberculosis (TB)	***	- clinical examination - microscopy of sputum - pulmonary X-ray - culture	- light microscope, acid-fast stain - TB laboratory facilities	* * *
2.2. Mycobacterium avium complex	*	- culture		*
2.3. Bacterial pneumonia	**	- clinical examination - pulmonary X-ray - culture (nasopharyngeal, sputum or blood)	- standard bacteriological laboratory facilities	* *
2.4. Bacterial gastroenteritis	**	- clinical examination - culture (stool) - stool microscopy (for exclusion of parasitic infections)	- light microscope	*

Disease	Frequency ^c	Mode of diagnosis	Equipment and reagents	Priority ^b
3. Viral Infections				
3.1. Cytomegalovirus (CMV) Ocular infection	*	- clinical examination - ophthalmological examination	- ophthalmoscope - (ophthalmological microscope)	*
central nervous system infection		- PCR of CSF	- PCR equipment	(*)
gastrointestinal infection		- endoscopy with biopsy - histopathological examination of biopsy material	- endoscopy equipment (rectoscope) - light microscope, staining material	* *
3.2. Varicella zoster virus (VZV)	*	- clinical examination - antigen detection by immunofluorescence (IF) - viral culture	- immunofluorescence microscope, reagents - virological laboratory facilities	*
3.3. Herpes simplex virus (HSV)	**	- clinical examination - IF - antibody detection - histopathologic examination of biopsy material - PCR - viral culture		* (*)
3.4. Progressive multifocal leucoencephalopathy (PML)	*	- clinical examination - PCR of CSF (JC virus) - CT - MRI - brain biopsy histology (exceptional)		* * (*)
4. Fungal infections				
4.1. Candida	***	- clinical examination - microscopy of plaque scrapings - endoscopy - X-ray (e.g. oesophagus)	- light microscope	* (*) (*) (*)

Disease	Frequency	Mode of diagnosis	Equipment and reagents	Priority
4.2. <i>Cryptococcus neoformans</i>	**	<ul style="list-style-type: none"> - serum antigen detection - CSF antigen - culture - histopathological examination of biopsy material 	<ul style="list-style-type: none"> - laboratory facilities for mycological cultures - mucicarmine stain, India ink stain 	<ul style="list-style-type: none"> (*) (*)
4.3. <i>Histoplasma capsulatum</i>	*	<ul style="list-style-type: none"> - serum antigen detection - histo- or cytopathological examination of biopsy material, sputum or lavage specimens - culture 		(*)
4.4. <i>Coccidioides immitis</i>	*	<ul style="list-style-type: none"> - serum antigen detection - histopathological examination of biopsy material - culture 		(*)
5. Other diseases				
5.1. Kaposi's sarcoma	**	<ul style="list-style-type: none"> - clinical examination - histopathologic examination of biopsy material 		*
5.2. Lymphomas	*	<ul style="list-style-type: none"> - clinical examination - cyto- or histopathological examination of biopsy or cell material - Ultrasonography - CT - MRI 	<ul style="list-style-type: none"> - Equipment for ultrasonography 	
5.3 HIV wasting syndrome	**	<ul style="list-style-type: none"> - clinical examination 		*
5.4 Other HIV related neurological complications	*	<ul style="list-style-type: none"> - clinical examination 		*

a) Clinical examination also includes clinical history and, ideally, routine blood samples such as full blood counts and inflammatory parameters.	BAL, Bronchoalveolar lavage
b)* Minimum (basic) requirement	CT, Computed Tomography
(*) Additional requirement	MRI, Magnetic Resonance Imaging
	PCR, Polymerase Chain Reaction
c) *** common	
** less common	CSF, Cerebrospinal fluid
* rare	
(N.B. May vary by geographical region or population group)	

DETERMINATION OF CD4+ T-CELL COUNTS

Determination of CD4+ T-cell counts has for many years been the conventional method for assessing the immune status of HIV infected persons. Low levels of CD4+ T-cells (<200 cells/cmm) predict decreased survival and increased risk of acquiring opportunistic infections. More recently the introduction of methods for assessment of plasma viral RNA levels has provided an alternative and complement to CD4+ T-cell measurements. Since plasma viral load is also a good predictor of clinical progression of the HIV disease, the need for regular CD4+ T-cell measurements may decrease. However, determination of CD4+ T-cell counts is still widely used, often in parallel with plasma viral load determinations. The extent to which CD4+ T-cell counts and plasma viral load measurements should be used and how the two methods can complement each other, is still to be determined.

In industrialised countries, the standard method for performing CD4+ T-cell counts in HIV-infected people is by flow cytometry. The method for flow cytometric immunophenotyping of peripheral blood samples using two-, three-, or four-colour monoclonal antibody panels is highly standardised and results are generally accurate and reliable. Most laboratories measure absolute CD4+ counts combining the results of two (or three) different laboratory techniques: the proportion of CD4+ T-lymphocytes is determined by flow cytometric analysis whereas the lymphocyte count (or the white blood cell count and the differential) is measured by haematological testing. Thus, each of the techniques used contributes to the accuracy of the final results.

Recently, several methods have been developed to derive absolute CD4+ T-cell counts directly from flow cytometric analysis. Such methods may yield less variable results but the accuracy must be validated against the laboratory's currently used conventional method. Laboratories performing CD4+ T-cell counts should follow published guidelines as well as manufacturers' instructions, and participate in performance evaluation programmes (11).

Flow cytometric instruments can today be purchased from three different manufacturers at a cost of around US\$ 40,000 - 80,000. These instruments require highly trained personnel and are thus unsuitable for routine use in laboratories with limited facilities. In such places, specimens may be transported to a central laboratory if they can be maintained at room temperature during transport and if they can be processed within acceptable time limits. A specimen for flow cytometric immunophenotyping must be processed within 48 hours of sample collection. Haematological testing must be performed within the time limit for which the haematology instrument has been validated, usually varying from less than 6 to 30 hours

the haematology instrument has been validated, usually varying from less than 6 to 30 hours after specimen collection. Immunophenotyping and haematological testing can thus be carried out at different laboratories. If specimens cannot be transported to laboratories at required temperatures or within acceptable time limits for flow cytometric analysis, e.g. in developing countries, an alternative method for CD4⁺ T-cell enumeration may be used.

Alternative methods for monitoring CD4⁺/CD8⁺ T-lymphocytes are summarised in Table 3. Alternative assays were reviewed in a WHO workshop in 1992. Results of a WHO collaborative multicentre field evaluation of alternative CD4 quantification techniques and other studies have demonstrated the reliability of these techniques and their potential for use in settings with limited facilities. A variety of assay principles exist, including commercial kits as well as in-house systems based on immunological reagents also available commercially. Some assays are designed to detect CD4⁺ cells only, whereas others also detect CD3⁺ and CD8⁺ cells. The various assay principles include detection of lymphocytes through labelling with monoclonal antibodies (mab) and then visualisation by colour reagents, either directly conjugated to the mab (FACSCount, Cytosphere, the immunoalkaline phosphatase technique) or indirectly via lysis of the mab labelled cells (Dynabeads). In the latter case the cells are visualised by trypan blue staining of the cell nuclei remaining after lysis. Alternatively, the CD4 and/or CD8 molecules may be detected by ELISA techniques, either directly on the cells (Capcellia, Zymune) or after releasing of the cell surface molecules through solubilization treatment (TRAx CD4). Apart from FACSCount, which requires a special instrument, these techniques can be applied without sophisticated instrumentation; Cytosphere, Dynabeads and the immunoalkaline phosphatase technique are read in a light microscope while Capcellia, Zymune and TRAx CD4 require instruments for ELISA analysis. The correlation between the alternative methods and flow cytometry is generally high, although in some studies variable correlation coefficients have been observed particularly for the ELISA based techniques when testing African samples. When comparing the various methods with flow cytometry the correlation coefficients may be high but the values obtained may be consistently too low or too high. It is, thus, important to evaluate and standardise the method chosen, in the local environment.

The choice of method will vary from one setting to another since each meets different laboratory requirements (Table 3). For example, methods based on the microtitre enzyme immunoassay technique would be suitable for laboratories which handle a large number of specimens but which have few staff. Such assays are also suitable for specimens collected from remote sites since they allow samples to be frozen and shipped in batches for testing. Methods based on cell counting using a microscope would be of use in laboratories which process few samples and have few staff. In general, the alternative methods require lower initial investments and are cheaper to perform than conventional flow cytometry. The price of the reagents needed to test one sample using an alternative method is about US\$ 5-10, with the exception of FACSCount and Capcellia which are more expensive. The corresponding cost using a flow cytometric method is US\$ 10-15. However, the alternative assays are less flexible and often more time consuming which is a constraint in large scale investigations.

When using the alternative methods for follow-up of patients it should be noted that results are likely to be more consistent if the same method is used each time. It is therefore recommended that laboratories use one type of assay. If a change is necessary it should be evaluated locally in parallel with the previous assay in order to make correct comparisons of

the results over time. Quality control programmes should also be set up and form part of the assay protocol.

In conclusion, the conventional and most reliable method for accurate CD4+ T-cell counts, especially when carried out large-scale, is flow cytometry. If flow cytometric analysis is not available, a number of alternative methods are available. These alternative methods meet different laboratory requirements and suit different settings. The initial investment and the cost of maintenance of equipment are substantially higher for flow cytometry testing as compared to alternative methods. These costs and subsequent expenditures for reagents for each tested sample are thus dependent on what method is used but do not vary much between different countries. Requirements for trained personnel and labour intensity largely depend on the method and thus, the labour costs will vary between different countries. The accuracy of the assay used has to be monitored and this is especially important when an alternative method is chosen.

Table 3. Summary of main characteristics of seven alternative methods for CD4/CD8 lymphocyte determination and flow cytometry.

	Flow cytometry	FACSCount™	Cytosphere	TRAx™CD4	Zymune	Dynabeads®	Capcellia	Immunokaline Phosphatase
Manufacturer	Becton Dickinson Coulter Corp. Ortho Diagnostic Systems	Becton Dickinson	Coulter Corp.	T Cell Diagnostics	Zynaxis Inc	Dynal A/S	Sanofi Diagnostics Pasteur	In-house assay with commercially available reagents
Instrument	Flow cytometer and computer assisted analysis. Preparation can be automated. May need use of cell counter.	Automated special instrument	Automated or light microscope and hemato-cytometer	Microtiter EIA	Microtiter EIA	Magnetic particles and counting equipment* or light microscope	Microtiter EIA	Light microscope
Detection system	Fluorescence labelled MAB against cell surface molecules	Fluorescence labelled anti-CD3, CD4 and CD8 MAB	Beads conjugated to anti- CD4 MAB	anti-CD4 and CD8 MAB	anti-CD4 and CD8 MAB	Magnetic beads conjugated to anti-CD4 and CD8 MAB	anti-CD4 and CD8 MAB	Slide based staining with anti-CD3, CD4 and CD8 MAB
Specimen	Whole blood with RBC lysis	Whole blood	Whole blood	Whole blood	Whole Blood	Whole blood	PBMC	Blood smear
Results	Double, triple or quadruple stainings of any cell surface marker where MAB are available	CD3, CD4 CD8 counts	CD4 counts	CD4 cell no. equivalent	CD4 and CD8 counts	CD4 and CD8 counts	pmol CD4/L and CD8/L	CD3, CD4, CD8 counts
Precision (Coeff variation)	< 5 > 15 % (depending on method used)	4 %	<5 %	<8 %	5 %	<10 %		95 % CI ±8-14
Correlation with flow cytometry (Correlation coeff)	NA	0.93-0.98 (several international studies)	0.74-0.91 (several international studies)	0.63-0.91 (several international studies)	0.92-0.94 (two studies in USA)	0.94 (one study only)		0.96 (one study only)
Cost (instruments, USD)	40 - 80 000	20 000	2 000	15 000	15 000	2 000	15 000	2 000
Cost/test (reagents only; USD; approx.)	10-15	20	8	6		5	28	3
Advantages	Powerful and flexible Allows for double,	Few steps, less human error	Simple and rapid	Simple Long sample shelf	Simple Can process many	Simple and rapid	Gives CD4 and CD8 counts	Low cost Long specimen shelf life

triple or quadruple stainings Can be used for examination of difficult samples where alternative techniques often are insufficient	Low biohazard risk Quick results Gives CD3, CD4 and CD8 counts	Can process many samples at a time	samples at a time Gives CD4 and CD8 counts	Gives CD4 and CD8 counts Flexibility in counting Small sample volume	Small sample volume
Disadvantages Expensive equipment and reagents Complex, requires highly trained personnel	Expensive equipment and reagents Work station allows only 8 samples at a time	Pipetting viscous samples difficult Gives CD4 counts only	Many pipetting steps	Few samples processed at a time. Subjectivity in visual counting. Short specimen shelf life. Not available as a kit	Laborious manual counting Complicated staining process
	Short shelf life Gives only CD4 counts			Expensive	

*) Automated cell counter or hemacytometer; PBMC - Peripheral blood mononuclear cells; Mab - Monoclonal antibodies; EIA - Enzyme immunoassay; CI - Confidence interval; RBC - Red blood cells; NA - Not applicable.

Viral load monitoring

Background

Our understanding of the pathogenesis of AIDS has greatly benefited from two recent therapeutic and methodological breakthroughs, namely the development and use of highly active antiretroviral combination therapy and the quantification of HIV-1 viral RNA in plasma. It has been shown that plasma levels of virus decrease very rapidly after the initiation of antiretroviral treatment. Typically, plasma HIV-1 RNA levels fall around 2 logs in the first two weeks of treatment. This rapid decline is followed by a slower second phase of decline.

In industrialised countries, quantification of human immunodeficiency virus type 1 (HIV-1) RNA in plasma has rapidly become a widely used method for follow-up of HIV-1 infected individuals. It is considered a crucial element of clinical management for assessing prognosis and effectiveness of therapy. This is based on the fact that plasma HIV-1 RNA levels appear to reach a set-point shortly after primary infection and that this set-point level strongly correlates with the subsequent risk of clinical progression. Moreover, early changes in RNA levels in response to treatment appear to predict long-term clinical benefit. In industrialised countries, the large scale use of quantitative HIV-1 RNA measurements has been greatly facilitated by the development of standardised, commercial kits.

Available methods

At present three commercial assays are available for the quantification of HIV-1 RNA in plasma, namely the HIV monitor assay (Roche), the Nuclisens HIV-1 QT (NASBA, Organon) and the Quantiplex HIV-RNA assay (bDNA, Chiron). The main characteristics of these assays are summarised in Table 4. These assays are under continuous development and new protocols which enable quantification down to around 50 copies of RNA per ml plasma and better detection of all genetic subtypes of HIV are currently being distributed. The cost per test (from the three companies) is officially a little less than US\$100. In-house methods for quantification of HIV-1 RNA have been described, but cannot be generally recommended mainly because within and between run variation is difficult to assess. A new improved method for quantification of HIV-1 p24 antigen in plasma exists but this assay has not yet been as extensively evaluated as the RNA assays. However, it holds potential for a less costly alternative in settings with limited resources.

HIV monitor (Roche)

The HIV monitor assay is based on the polymerase chain reaction (PCR) technique. Viral RNA is extracted from 200 µl of plasma and following reverse transcription PCR the HIV-1 RNA copy numbers are determined by an ELISA-like method. The assay takes approximately 8 hours to run. The lower limit of detection is 200-500 HIV-1 RNA copies per ml plasma, but a modified assay with a lower limit of detection (50 copies per ml) is being developed. This modified assay requires ultracentrifugation of 500 µl plasma prior to RNA extraction. The first (current) version of the assay gives false low results with samples from many subtype A and E infected persons, but new versions (add-in primer and version 1.5) of the assay with improved detection of HIV-1 group M subtypes are becoming available. However, HIV-1 group O and HIV-2 are not detected. Equipment includes a thermocycler (cost approximately US\$10,000) an ELISA photometer (cost approximately US\$15,000) and for the ultrasensitive technique, an ultracentrifuge (cost approximately US\$25,000).

Nuclisens HIV-1 QT; NASBA (Organon)

The Nuclisens HIV-1 QT is based on the NASBA (nucleic acid sequence based amplification) method, which is an isothermic amplification method which uses three enzymes (reverse transcriptase, RNase H and T7 RNA polymerase). The viral RNA extracted from plasma is NASBA amplified and the detection system is based on electrochemoluminescence (ECL) which requires a special apparatus. The assay takes approximately 12 hours to run. The lower limit of accurate quantification is dependent on the sample volume used, with 200 µl of plasma input the detection limit is 400 copies/ml. However, up to 2 ml plasma can be used resulting in a detection limit of 40 RNA copies/ml plasma. The first (current) version of the assay gives false low results with samples from many subtype A and E infected people. HIV-1 group O and HIV-2 is not detected. Equipment required includes a heat-block (cost approximately US\$3,000) and special detection apparatus (Table 4).

Quantiplex HIV-RNA assay; bDNA (Chiron)

The bDNA assay differs from the other two assays in that it is based on signal amplification rather than target amplification. The assay has an ELISA like format. Virus is concentrated from 1 ml of plasma by ultracentrifugation and viral RNA is released in a buffer which contains multiple HIV-1 probes. The HIV-1 RNA (with attached probes) is allowed to bind to microplate wells coated with HIV-1 specific capture probes. Enzyme labelled detection probes (branched DNA) and a chemiluminescent substrate are subsequently added and the light output is measured in a special chemoluminescensometer. The assay takes approximately two days to run. The lower limit of accurate quantification is 500 copies/ml and around 100 copies/ml in a revised assay. The assay appears to be able to detect all genetic subtypes within group M with equal efficiency. HIV-1 group O and HIV-2 are not detected. Equipment required includes an ultracentrifuge (cost approximately US\$25,000), and the special detection apparatus (Table 4).

Comparison of the methods

The three commercially available assays are very similar in terms of cost, labour, sensitivity and precision. Thus, no single assay can be considered superior. There are, however, small differences between the assays which may be important in some situations. The equipment needed for the Roche assay is often already at hand in the laboratory, but the special apparatus needed for the other two assays can probably be obtained for free or at low cost from the manufacturers, at least if large sample series are analysed. Furthermore, the more extensive special set-up for the Organon and Chiron assays makes them more suitable for large scale analysis. The somewhat longer time required to complete the Organon and Chiron assays is mainly due to longer incubation times. In most countries, except possibly the USA, many genetic subtypes coexist; thus, assays which do not detect all subtypes with equal efficiency, i.e. the first generation Roche and Organon assays, should probably be avoided.

It should be noted that for all three assays computer equipment is also required.

Options for different settings

There are really only two options: to use or not to use HIV-1 RNA assays for routine monitoring of HIV-1 infected individuals. The cost of the assays, equipment and a suitable laboratory facility are the major obstacles. In addition, well trained technicians are a prerequisite for a meticulous and accurate use of the assays. However, in relation to the cost of modern antiretroviral combination therapy with protease inhibitors, the cost of HIV-1 RNA quantification is moderate. It can be argued that RNA quantification should be used in settings where combination therapy is given. It is likely that monitoring of HIV-1 RNA levels before and during treatment will be cost-effective, because it will

give information about when treatment should be started, stopped or changed. Recommendations about how HIV-1 RNA levels should be used in routine patient care have been published. In untreated patients HIV-1 RNA levels are usually measured every four to six months. After start of treatment, measurements are usually performed every three months and often also one month after start or change of treatment.

Although ideally viral load monitoring should be available when prescribing HAART, in some resource poor settings it will not be available and monitoring of disease progression and response to treatment will be by clinical indicators and CD4 measurement.

Table 4. Summary of main characteristics of assays for viral load monitoring

Method	Principle	Detection limit	Comments	Special equipment needed	Cost (US\$)
HIV Monitor (Roche)	RT-PCR followed by ELISA-like method to determine copy numbers	Standard: 200-500 HIV-1 RNA copies/ml plasma Ultrasensitive: 50 copies/ml	Equipment often available for other purposes, in standard virological laboratories First generation assay gives false low results with many subtype A and E samples HIV-1 group O and HIV-2 are not detected	- thermocycler - ELISA photometer - ultracentrifuge	10 000 15 000 25 000
Nuclisens HIV-1 QT; NASBA (Organon)	Isothermic amplification of extracted RNA, using three enzymes (NASBA). Electrochemoluminescence (ECL) based detection system	Standard: (sample volume 200 µl): 400 HIV-1 RNA copies/ml plasma Using 2 ml plasma: 40 copies/ml	First generation assay gives false low results with many subtype A and E samples Special detection apparatus needed HIV-1 group O and HIV-2 are not detected	- special detection apparatus - heat block	negotiable 3 000
Quantiplex HIV-RNA assay; bDNA (Chiron)	Signal amplification through enzyme labelled detection probes. Chemoluminescence based detection system	Standard: 500 HIV-1 RNA copies per ml plasma Revised: 100 copies/ml	Detects all HIV-1 group M strains Does not detect HIV-1 group O or HIV-2. Special detection apparatus needed	- Ultracentrifuge Special detection apparatus (chemoluminescence meter)	25 000 negotiable

Monitoring of resistance of HIV

Background

HIV is characterised by a high level of genetic variability and rapid evolution which gives it an extraordinary ability to respond to environmental changes such as that induced by antiretroviral therapy. As a consequence development of resistance is the major obstacle to long-term effective treatment.

Resistant virus variants have been documented to virtually all antiretroviral drugs which are in use or under development. Resistance can develop within weeks after onset of treatment with single antiretroviral drugs, such as the non-nucleoside reverse transcriptase inhibitors (NNRTI) and 3TC, because of the rapid turn-over of HIV-1 *in vivo*. The speed with which resistance develops is in part determined by the number of mutations required for full resistance. For some drugs, such as those mentioned above, a single point mutation is sufficient for complete resistance. In such cases resistant variants are usually present, albeit at low level, within the pre-treatment virus population. Resistance to other drugs or drug combinations require multiple mutations. Such variants do not usually pre-exist, but instead evolve as the virus consecutively acquires more and more mutations. To avoid development of resistance it is important to use drugs which cannot be circumvented by single mutations and which are potent enough to block virus replication and thereby the risk of accumulation of mutations. These two goals can in principle only be achieved by simultaneous use of three or more drugs, i.e. so called highly active antiretroviral treatment (HAART).

There are several different methods for determining the sensitivity of HIV to antiretroviral drugs, but no simple standardised method for routine use. All available methods are relatively complex and furthermore the interpretation of the assays is complicated. Thus, the exact consequence of development of resistance is not fully known. It is clear that resistance often leads to loss of treatment effect, but the loss is not always complete.

Although the monitoring of drug resistant variants is important, especially with more widespread use of ARVs, techniques are currently complex and expensive and their use will be confined to reference centres.

Available methods

Broadly, there are two techniques for determining antiviral sensitivity: biological and molecular techniques. Each of these has advantages and drawbacks. An advantage of the biological assay is that the drug sensitivity of a virus isolate is measured directly, whereas for the molecular assays it is inferred from the presence or absence of mutations known to confer drug resistance. This may be far from straightforward in patients who display complex mutational patterns after treatment with several different antiretroviral (ARV) drugs. However, the biological assays require access to a virus isolate. This in itself is a problem because the isolation process is known to select for certain variants from the population present *in vivo* (28). Furthermore, the isolate used is usually obtained from peripheral blood mononuclear cells (PBMC) and may therefore not be fully representative of the actively replicating virus population which is better represented in plasma. These problems can be circumvented with the molecular assays because they allow direct analysis of plasma virus.

Biological methods for determination of drug susceptibility

The classical way to determine antiviral sensitivity is to isolate virus and test its sensitivity by culture in the presence of different concentrations of the antiretroviral drugs in question. It is customary to express the results as the concentration of drug required to inhibit virus replication by 50% or 90% (i.e. 50% and 90% inhibitory concentration, IC_{50} and IC_{90}). There exist "standardised" protocols for the biological antiviral sensitivity assay such as the ACTG protocol, but these standardised protocols require a lot of manual laboratory work and should not be regarded as kits. Thus, the assay is labour intensive and usually takes a minimum of one month to complete an analysis, even if the actual hands-on time is much less. The method also requires that virus be isolated, which is not always possible. The method requires access to a laboratory facility fully equipped for work with infectious HIV, i.e. biosafety level 3 (BSL3) facility which limits the possibilities for large scale use.

Molecular methods for determination of drug susceptibility

There are a number of published molecular methods for determination of drug susceptibility which can be broadly divided into point mutation assays and sequencing strategies.

Point mutation assays: Commonly used methods to detect point mutations known to be associated with drug resistance include primer specific PCR and a type of mini-sequencing. A commercial point mutation assay based on the line-probe technique (LiPA HIV-1 RT, Innogenetics Murex) has recently been introduced. The major advantage of the point mutation assays is that they can analyse the replicating virus population (i.e. plasma virus) and that they are comparably easy to perform. The necessary equipment is usually limited to a thermocycler for PCR (cost approximately US\$10,000). A draw-back is that the assays can only be used to detect predefined mutations.

DNA sequencing: There exist a number of sequencing strategies which can be used to detect the mutations which confer drug resistance. Automated systems such as those supplied by Applied Biosystems or Pharmacia-UpJohn are often used. The "gene chip" technology can also be used. The advantage of the sequencing strategies over the point mutation assays is that they can be used to identify new mutations, in addition to predefined mutations. The sequencing methods are labour intensive and require access to expensive equipment.

Recombinant virus assay

The recombinant virus assay is a third type of assay which combines the biological and molecular assay. In this assay, the gene fragments of interest are amplified from plasma by PCR and co-transfected into permissive cells with a HIV plasmid from which the corresponding parts of the gene of interest have been deleted. Infectious virus, which is reconstituted by homologous recombination, is harvested and tested in a biological assay. The assay is attractive because reverse transcriptase and protease sequences obtained from plasma can be biologically tested on a neutral viral backbone sequence. However, the assay requires a highly developed BSL3 facility in which recombinant viruses can be generated and tested.

Options for different settings

As outlined above there exist several different methods for determination of the drug susceptibility of HIV. At present these assays cannot be recommended for large scale routine use because the interpretation of assays is complicated and because the clinical consequences of resistance are not fully understood. However, drug susceptibility assays are very important tools in clinics and laboratories actively involved in research on antiretroviral treatment. Even in these settings the choice of antiretroviral regimen to individual patients usually becomes a pragmatic decision influenced by

plasma HIV-1 RNA measurements, prior treatment as well as treatment side effects and compliance. Accordingly, drugs susceptibility assays, in contrast to HIV-1 RNA assays, are not advocated in the updated recommendations for antiretroviral therapy of the International AIDS Society-USA Panel (27).

SUMMARY

The laboratory requirements for support of ARV treatment can be set at different levels depending on local circumstances: the epidemiological situation, resources available, existing facilities and the number of staff and their level of training. Providing laboratory services for the safe and effective use of ARV treatment requires a degree of centralisation. While many methods are relatively inexpensive and straightforward, others are highly specialised and require large investments in equipment and well trained staff. A country with a relatively small population may choose to have one centre specialised in the back-up for ARV treatment. In larger countries regional centres may be needed, perhaps complemented by a national reference centre. If these services do not already exist it is recommended that a reliable system be set up in one centre before decentralisation. Small and/or resource poor countries may consider international collaboration in order to meet the necessary requirements. Although the crucial HIV quantification techniques are highly specialised and costly, it is important to bear in mind that much of the back-up and follow-up of ARV treatment can be carried out with simple and well-known method requiring a moderate level of instrumentation.

Facilities have to be available for correct diagnosis of HIV infection, monitoring of CD4 levels in patients, side effects of ARV treatment, identification and diagnosis of opportunistic infections, viral load monitoring and observation of resistance of HIV.

In a setting with limited resources, it is recommended that diagnosis of HIV infection and CD4 T-cell counts be done with alternative methods which are less costly in terms of equipment as well as reagents. These techniques are generally more simple to perform and the analyses required are less complicated. A majority of the adverse reactions to ARV treatment can be monitored by careful clinical examination and a set of basic laboratory tests, such as blood counts including differential, bilirubin and liver enzymes, amylase, urate and glucose (simple dip tests of urine are usually sufficient). Similarly, for appropriate monitoring of opportunistic infections, a well trained and experienced clinician can do this with the assistance of chest X-ray and light microscopy. The latter will include (the relatively inexpensive) reagents for staining of specimens for microbiological diagnosis, e.g. pneumocystis carinii, tuberculosis and intestinal parasites. Monitoring of plasma viral load is an important part of ARV treatment. However in some developing countries CD4 counts will be used for monitoring disease progression and response to ARVs. Viral load provides information on treatment adherence and the development of resistance. The currently available method are expensive and complex but in relation to the cost of the treatment, not preposterous. Less costly and simpler alternatives may be available in the future. The use of CD4 counts, for example, with an alternative method may give useful information but cannot completely replace viral load measurements. It should not be a priority to purchase equipment for detailed analysis of resistance patterns in situations of limited resources.

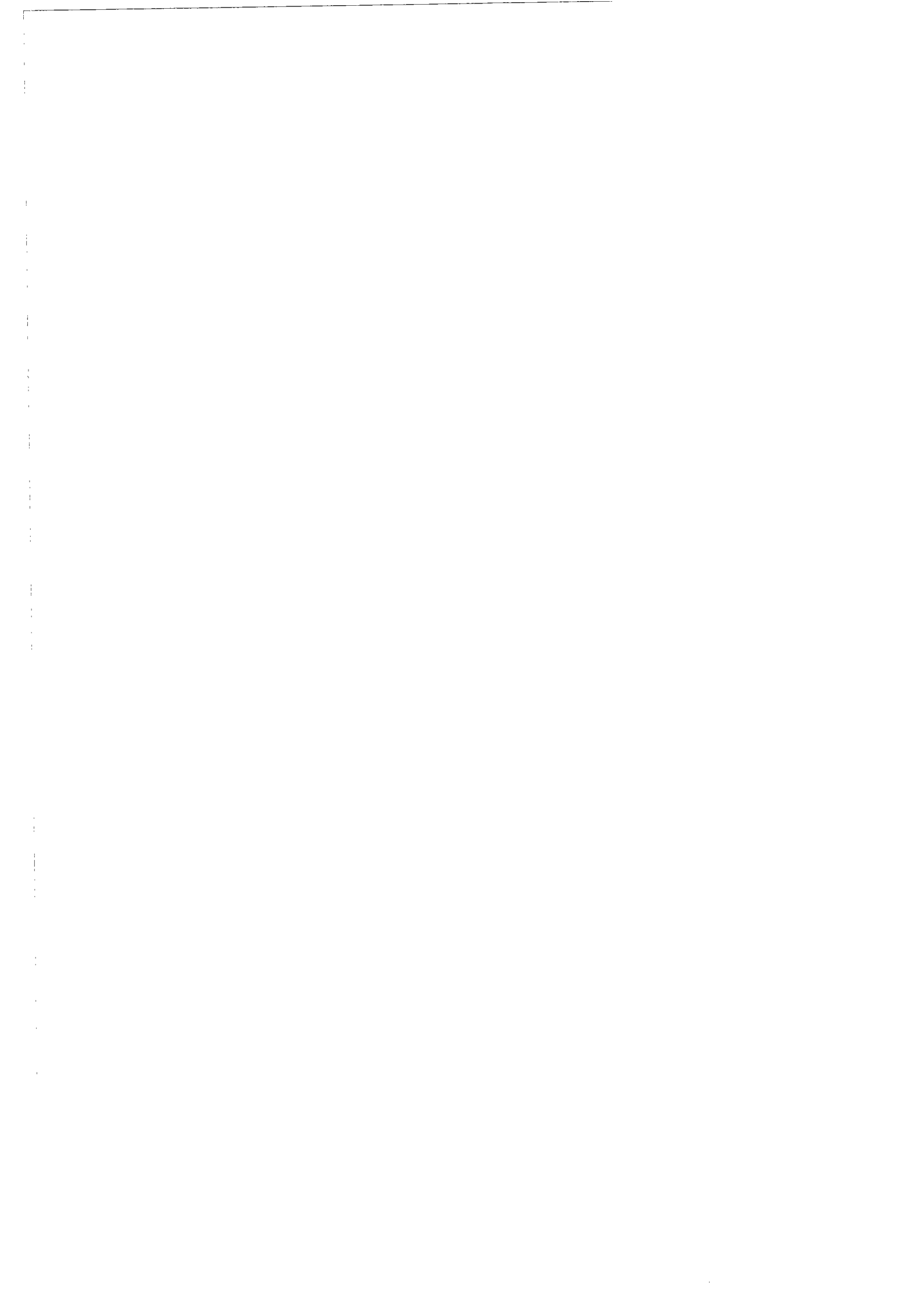
In a setting with a higher level of resources, it will be possible to establish more detailed diagnostic procedures. The addition of endoscopy equipment for example, for gastrointestinal infections, bronchoscopy for pneumocystis carinii, bacteriological cultures including tuberculosis, computed tomography (CT) for cerebral toxoplasmosis, polymerase chain reaction (PCR) of cerebrospinal fluid (CSF) for progressive multifocal leucoencephalopathy (PML) and

immunofluorescence for herpes simplex virus infections, will increase the specificity in the diagnosis of opportunistic infections and, thus, lead to more efficient and cost effective treatment of these complications of HIV infection. Determinations of T lymphocyte subsets will benefit from the addition of flow cytometry which is more powerful and flexible than the alternative methods for CD4/CD8 analyses. Alternative methods may be used by smaller units, e.g. at regional centres, while a national reference centre may have flow cytometry equipment. This implies that the different methods have been evaluated and standardised against each other. Monitoring of viral load should be part of the routine services. Which method for viral load measurement to choose is more a matter of available local equipment, experience and preference than differences in performance. It should, however, be noted that the first generation of the Organon and Roche assays may give false low results on subtype A and E samples. The establishment of some type of detailed analysis of ARV resistance may be considered, if not sought through international collaboration.

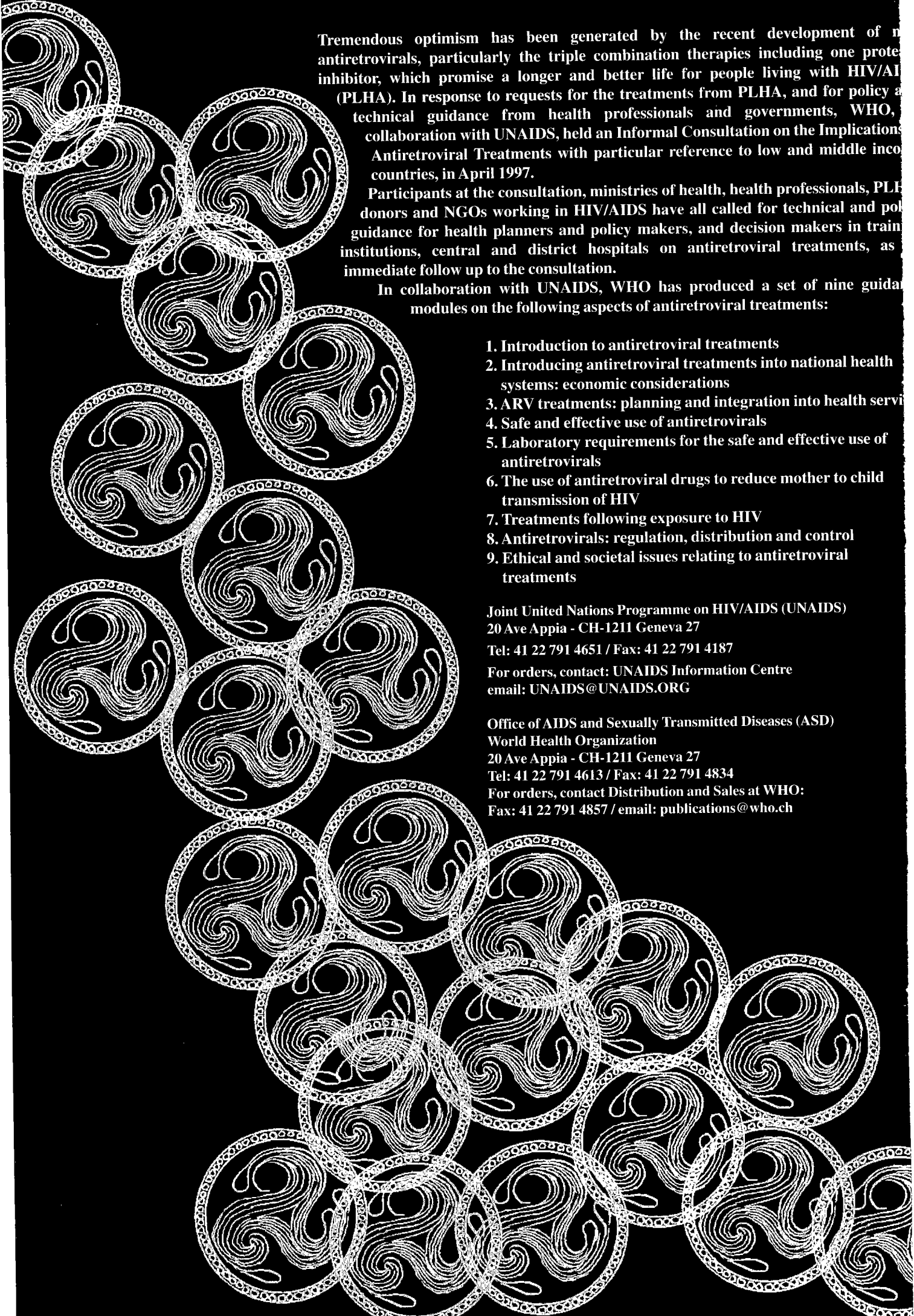
REFERENCES

1. Joint United Nations Programme on HIV/AIDS (UNAIDS)/WHO. Revised recommendations for the selection and use of HIV antibody tests. *Weekly Epidemiol Rec* 1997;72:81-88.
2. Andersson S, da Silva Z, Norrgren H, Dias F, Biberfeld G. Field evaluation of alternative testing strategies for diagnosis and differentiation of HIV-1 and HIV-2 infections in an HIV-1 and HIV-2-prevalent area. *AIDS* 1997;11:1815-22.
3. Lyamuya E, Bredberg-Råden U, Massawe A, et al. Performance of a modified HIV-1 p24 antigen assay for early diagnosis of HIV-1 infection in infants and prediction of mother-to-infant transmission of HIV-1 in Dar es Salaam, Tanzania. *J Acquir Immune Defic Syndr and Human Retrovir* 1996;12:421-426.
4. Sninsky J, Kwok S. The application of quantitative polymerase chain reaction to therapeutic monitoring. *AIDS* 1993;7 (suppl 2):S29-34.
5. De Vita Jr V, Hellman S, Rosenberg S (editors). *AIDS: etiology, diagnosis, treatment and prevention*, 4th ed. Lippincott-Raven Publishers, Philadelphia, USA, 1997.
6. van Praag E, Fernyak S, Martin Katz A (editors). *The implications of antiretroviral treatments. Informal consultation, April 1997.* World Health Organization in collaboration with UNAIDS, Geneva. WHO/ASD/97.2.
7. Centers for Disease Control and Prevention. 1997 Revised guidelines for performing CD4+ T-cell determinations in persons infected with human immunodeficiency virus (HIV). *MMWR* 1997;46(No. RR-2):1-30.
8. Nicholson JA, Velleca WM, Jubert S, Green T, Bryan L. Evaluation of alternative CD4 technologies for the enumeration of CD4 lymphocytes. *J Immunol Methods* 1994;177: 43-54.
9. Lyamuya EF, Kagoma C, Mbena E, Urassa W, Pallangyo K, Mhalu FS, Biberfeld G. Evaluation of the FACScount, TRAxTM CD4 and the Dynabeads methods for CD4+ T lymphocyte determinations. *J Immunol Methods* 1996;195:103-112.
10. Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P, Lifson JD, Bonhoeffer S, Nowak MA, Hahn BH, Saag MS, Shaw GM. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 1995;373:117-22.
11. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 1995;373:123-6.
12. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Science* 1996;271:1582-6.

13. Carpenter CCJ, Fischl MA, Hammer SM, Hirsch MS, Jacobsen DM, Katzenstein DA, Montaner JSG, Richman DD, Saag MS, Schooley RT, Thompson MA, Vella S, Yeni PG, Volberding PA. Antiretroviral therapy for HIV infection in 1997. Updated recommendations of the International AIDS Society - USA panel. *JAMA* 1997;277:1962-9.
14. Boni J, Opravil M, Tomasik Z, Rothen M, Bisset L, Grob PJ, Luthy R, Schupbach J. Simple monitoring of antiretroviral therapy with a signal-amplification-boosted HIV-1 p24 antigen assay with heat-denatured plasma. *AIDS* 1997;11:F47-52.
15. Alaeus A, Lidman K, Sönnnerborg A, Albert J. Subtype-specific problems with quantification of plasma HIV-1 RNA. *AIDS* 1997;11:859-65.
16. Richman DD, Havlir D, Corbeil J, Looney D, Ignacio C, Spector SA, Sullivan J, Cheeseman S, Barringer K, Pauletti D. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. *J Virol* 1994;68:1660-6.
17. Schuurman R, Nijhuis M, van Leeuwen R, Schipper P, de Jong D, Collis P, Danner SA, Mulder J, Loveday C, Christopherson C. Rapid changes in human immunodeficiency virus type 1 RNA load and appearance of drug-resistant virus populations in persons treated with lamivudine (3TC). *J Infect Dis* 1995;171:1411-9.
18. Kaye S, Loveday C, Tedder RS. A microtitre format point mutation assay: application to the detection of drug resistance in human immunodeficiency virus type-1 infected patients treated with zidovudine. *J Med Virol* 1992;37:241-6.
19. Stuyver L, Wyseur A, Rombout A, Louwagie J, Scarcez T, Verhofstede C, Rimland D, Schinazi RF, Rossau R. Line probe assay for rapid detection of drug-selected mutations in the human immunodeficiency virus type 1 reverse transcriptase gene. *Antimicrob Agents Chemother* 1997;41:284-91.
20. Boucher CA, Keulen W, van Bommel T, Nijhuis M, de Jong D, de Jong MD, Schipper P, Back NK. Human immunodeficiency virus type 1 drug susceptibility determination by using recombinant viruses generated from patient sera tested in a cell-killing assay. *Antimicrob Agents Chemother* 1996;40:2404-9.







Tremendous optimism has been generated by the recent development of new antiretrovirals, particularly the triple combination therapies including one protease inhibitor, which promise a longer and better life for people living with HIV/AIDS (PLHA). In response to requests for the treatments from PLHA, and for policy and technical guidance from health professionals and governments, WHO, in collaboration with UNAIDS, held an Informal Consultation on the Implications of Antiretroviral Treatments with particular reference to low and middle income countries, in April 1997.

Participants at the consultation, ministries of health, health professionals, PLHA donors and NGOs working in HIV/AIDS have all called for technical and policy guidance for health planners and policy makers, and decision makers in training institutions, central and district hospitals on antiretroviral treatments, as well as immediate follow up to the consultation.

In collaboration with UNAIDS, WHO has produced a set of nine guidance modules on the following aspects of antiretroviral treatments:

1. Introduction to antiretroviral treatments
2. Introducing antiretroviral treatments into national health systems: economic considerations
3. ARV treatments: planning and integration into health services
4. Safe and effective use of antiretrovirals
5. Laboratory requirements for the safe and effective use of antiretrovirals
6. The use of antiretroviral drugs to reduce mother to child transmission of HIV
7. Treatments following exposure to HIV
8. Antiretrovirals: regulation, distribution and control
9. Ethical and societal issues relating to antiretroviral treatments

Joint United Nations Programme on HIV/AIDS (UNAIDS)
20 Ave Appia - CH-1211 Geneva 27

Tel: 41 22 791 4651 / Fax: 41 22 791 4187

For orders, contact: UNAIDS Information Centre
email: UNAIDS@UNAIDS.ORG

Office of AIDS and Sexually Transmitted Diseases (ASD)
World Health Organization

20 Ave Appia - CH-1211 Geneva 27

Tel: 41 22 791 4613 / Fax: 41 22 791 4834

For orders, contact Distribution and Sales at WHO:
Fax: 41 22 791 4857 / email: publications@who.ch