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Distribution: General

Surveillance of HIV Drug Resistance

Report of a Workshop, Pune, India, 7-11 August 2006

WHO Project: ICP BCT 001



**World Health
Organization**

Regional Office for South-East Asia
New Delhi

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1. Introduction

The AIDS epidemic continues to spread in the World Health Organization's South-East Asia (SEA) Region. With the exception of sub-Saharan Africa, this Region is most affected by AIDS in the world. About 40 million people throughout the world have been infected with the human immunodeficiency virus (HIV) that causes AIDS. Of these, as many as 7.8 million are in the SEA Region.

Different Member States have launched antiretroviral therapy (ART) programmes in the public sector. Given the nature of HIV-infection and the life-long treatment involved, some degree of HIV drug resistance (HIVDR) is bound to develop as a consequence. In untreated persons, up to 10 billion new viruses are produced daily and 100 million new cells are infected. Of the 10 billion new viruses, around one million will undergo a mutation which may make the virus resistant to ART drugs. If selection pressure is exerted by the same drug, the wild virus can be completely replaced within a month by the drug-resistant virus and cause primary resistance in a new individual. The selection pressure becomes greater when ART is not administered rationally and not fully adhered to.

The necessity of lifelong continuation of antiretroviral treatment makes its compliance very difficult. Several studies have shown that adherence to treatment is erratic in a large percentage of people. This increases the probability of development of resistance. Also, historically prescribed mono- and bi-therapy as well as planned or unplanned treatment and drug supply interruptions can accelerate the process of development of resistance.

The determination of resistance is possible only through laboratory-based analysis of viral isolates. Both genetic-based and virological methods are available. Establishment of infrastructure, performance of tests, quality systems and a mechanism of validation of results are new issues which require serious consideration. The linkages between the laboratories and national programmes are to be firmly developed so that National AIDS Programmes can benefit from the laboratory-based surveillance.

To discuss the issue of resistance in HIV and to orient the nationals on various techniques to determine it, a workshop was organized at the National AIDS Research Institute (NARI), Pune, India from 7–11 August 2006. Fifteen participants from nine Member States attended the workshop. Only DPR Korea and Timor-Leste expressed inability to nominate participants. The facilitators included the Director and senior staff of NARI, experts from the YRG Institute on HIV Care and the WHO staff. The list of participants and the workplan are at Annex 1 and 2 respectively.

2. Objectives

The objectives of the workshop were to:

- (1) Review the status of surveillance of drug resistance in HIV high-burden Member States.
- (2) Provide hands-on training on both genetic and virological assays for determination of resistance.
- (3) Identify requirements for establishment of infrastructure to undertake surveillance and sharing of information with national programmes.
- (4) Discuss participation of national laboratories in WHO network (HIVResNet).

3. Inaugural session

The workshop was inaugurated by Lieutenant-General S. Mukherjee, Director and Commandant, Armed Forces Medical College (AFMC), Pune, India. In his inaugural address, General Mukherjee highlighted the problem of HIV drug resistance (HIVDR) and the policy for ART in India. Referring to the growing HIV epidemic and need to use ART as an effective interventional tool, he pointed out some of the constraints including high costs, inadequate adherence and adverse effects that act as hurdles towards achieving success of ART regimens. He emphasized that determination of drug resistance in HIV is a part of the ART Guidelines and is an independent predictor of ART success. He stressed the importance of HIVDR surveillance for measuring the transmitted resistant strains and the

monitoring of resistance to ART in the HIV patients. He also advocated the need for collaboration between various institutions and sharing of experiences so as to minimize the emergence and spread of drug resistance in HIV.

Dr Rajesh Bhatia, Regional Adviser, Blood Safety and Clinical Technology with the WHO Regional Office for South-East Asia (SEARO), read out the address of Dr Samlee Plianbangchang, Regional Director, WHO SEA Region. Dr Samlee informed that HIV/AIDS has been identified as a disease of global importance under the Millennium Development Goals. The global community has set the target of halting the spread of the pandemic and even reversing it by 2015. ART has become an integral component of any strategy aimed to contain the burgeoning epidemic of HIV. WHO has been advocating the importance of preventing the infection as well as providing ART to the millions who need it.

Of the 900 000 people who are in need of ART currently, only 164 000 are being treated in countries of the SEA Region. The national ART programmes are being scaled up along with a plan to provide treatment to a large number of HIV-infected persons in the near future. Given the nature of HIV-infection and the need for life-long treatment, some degree of HIV drug resistance is inevitable. With a large number of people receiving ART, surveillance for resistance becomes critical. The surveillance for emergence of resistance in HIV against the drugs recommended by the National Programme provides critical inputs in reviewing the efficacy of the treatment regimens. Reliable data on resistance are essential to plan or modify the composition of ART in order to derive the maximum benefit from this intervention.

Emphasizing the role of laboratories in determination of resistance, Dr Samlee informed that this workshop shall play an important role in reviewing the status of resistance surveillance in countries of the SEA Region and to provide hands-on experience for strengthening laboratory-based monitoring of resistance. The workshop shall also address the issues of infrastructure requirement, implementation of quality systems, role of networking, and a mechanism for utilization of data which would help the National AIDS Programme to increase its efficacy in providing ART to all those who need it.

4. Proceedings of the workshop

The workshop included the presentation of country reports to review the existing capacity of the countries, presentations and discussions to disseminate new information, and hands-on experience in various technologies to determine resistance to HIV.

Country reports

Bangladesh

The reported number of HIV-positive individuals in Bangladesh is currently 465. However, it is estimated that around 13 000 people are living with HIV with a prevalence of less than 0.01%. Till date, 72 deaths have been reported in the country with AIDS as the underlying cause. The number of people on ART is not known. There is no national strategy on ART and laboratory facilities for drug resistance have not been established.

Bhutan

The national HIV Control Programme was initiated in 1998. Currently 90 people have been detected with HIV in Bhutan. Twelve patients have been on ART. The Public Health Laboratory and the Central Laboratory are jointly responsible for the close physical and clinical supervision of the patients through follow-up laboratory investigations and counselling. No facilities exist for HIV drug resistance testing.

India

India has one of the highest number of persons living with HIV/AIDS in the world today, although the overall prevalence remains low (<1%). As per estimates of the National AIDS Control Organization (NACO), there were 5.21 million Indians living with HIV at the end of 2005, of which 39% were females. According to the UNAIDS 2006 report, India has an estimated 5.7 million people living with HIV.

The Government of India is currently in the early stages of preparing for the third phase of the “National AIDS Control Programme” (NACP-3), for which a multi-disciplinary design team has been constituted for the

preparatory stages. The Government of India provides free ART. A total of 32 000 HIV-positive individuals are on ART in India. ART is also provided by the private sector but the number of people availing of these services is not known.

HIV drug resistance genotyping is being carried out in few institutes including National AIDS Research Institute (NARI), Tuberculosis Research Centre, Chennai, All India Institute of Medical Sciences, New Delhi, and YRG Care, Chennai. Usually commercial kits (Viroseq) and in-house methodologies are employed. Several staff members have received advanced training in genotypic drug resistance studies in US and French laboratories. NARI has been shortlisted among the four laboratories assessed by WHO for accreditation.

Future plans include the establishment of a surveillance system in India to monitor the spread of HIV drug resistance (HIVDR) in naïve and ARV-treated subjects and generation of HIV drug resistance database for treatment of naïve and treated subjects. A major constraint in undertaking full-fledged HIV resistance testing in India is the cost of commercially available HIVDR genotyping kits.

Indonesia

The estimated number of persons living with HIV varies between 90 000 to 130 000, of which around 30 000 are estimated to be eligible for ART. In 2004 the National Antiretroviral Programme was started in 25 hospitals providing 4000 patients with highly active antiretroviral therapy (triple drug combination) without protease inhibitors. In the year 2005, the programme was expanded to more than 75 hospitals, serving at least 5600 patients. The strategy and guidelines on ART have been developed and implemented by the Directorate-General of Communicable Disease Control and Environmental Health, Ministry of Health. This aims at 12 000 persons to be on ART by the end of 2006 and 27 000 by the end of 2007.

The Ministry of Health has decided to develop a working group for antiretroviral resistance in order to participate in the WHO HIV Drug Resistance Surveillance. Three laboratories in Jakarta were assessed for accreditation as a national laboratory by WHO for genotypic HIV drug resistance testing. However, none of the assessed laboratories fulfilled the WHO criteria. One of the laboratories, the Department of Microbiology,

Medical Faculty, University of Indonesia, continues to improve the capacity in performing HIV genotypic assay for Drug Resistance Testing by developing an in-house assay. This laboratory is also actively involved in the working group for National HIV-DRS that includes the development of Standard Operating Procedures (SOP) for collection and processing of specimens to be used for the WHO HIV Drug Resistance Surveillance.

Maldives

Maldives is highly vulnerable to the AIDS pandemic because of several reasons. Maldivians frequently travel abroad for purposes of business, education, recreation and medical treatment. Approximately 2000 young Maldivians are employed overseas as seamen. The number of persons addicted to drugs (both males and females) including intravenous drug users are increasing. There is a very high rate of divorce and subsequent remarriage. About 45% of the population is aged between 15 and 45 years. There are around 40 000 foreign migrant workers employed in the country, 90% of whom are males. There are also more than 600 000 tourist arrivals per year. The first case on HIV/AIDS in the country was detected in 1991. Since then, a total of 14 cases (three females and 11 males) have been detected. Nine of these cases which were diagnosed as AIDS cases have died. Of the three alive, one is on ART. This patient contracted HIV in 1994 and was diagnosed with AIDS in 2004. At present, he is on a treatment regime comprising of zidovudine, lamivudine, efavirenz and septran. There is no drug resistance monitoring system in the country and the need to develop such systems is being recognized.

Myanmar

AIDS has been identified as one of the three priority diseases in the National Health Plan in Myanmar. The National AIDS Programme (NAP) is responsible for the prevention and control of HIV/AIDS and sexually transmitted diseases. The NAP has 43 AIDS/STD teams (clinics) located strategically in 34 townships providing STD services.

The number of HIV-positive cases recorded and reported AIDS cases (cumulative till end-2003) in Myanmar is 53 015 and 7174 respectively. The cumulative number of deaths reported due to AIDS till end 2003 was

3324. An estimated 177 279 People Living With HIV/AIDS (PLWHA) were reported in Myanmar as on March 2002.

ART was started in Myanmar in 2003 at the Waibargy Specialist Hospital, Yangon. In June 2005, ART was also launched in public health hospitals. ART is being administered in six townships and four border towns with the total number of patients receiving it in Myanmar being 2500.

The available laboratory support includes HIV testing, opportunistic infection diagnosis and CD4 count estimation. Viral load estimation is being planned and equipment for this has been procured and training begun. There is no national laboratory facility for HIV drug resistance testing.

Nepal

The first case of HIV in Nepal was detected in 1988. The major route of transmission of infection is heterosexual. An estimated 70 000 people are living with HIV/AIDS in Nepal. There are 300 deaths (2002) annually among the HIV-infected population. The estimated national prevalence is 0.55% and all regions of the country are equally affected.

Antiretroviral therapy was launched in 2004. Currently it is being provided through six centres and future scaling up of ART programmes in major hospitals in a phased manner is being planned. The number of PLWHAs for whom drugs distribution was needed was 555 as on 31 March 2006 and the number of PLWHAs on ARV treatment was 357. **Laboratory facilities for HIV drug resistance** are currently not available in Nepal.

Sri Lanka

First indigenous transmission of HIV in Sri Lanka was reported in 1987. Till date there were 785 reported HIV-positive cases and 150 AIDS deaths in the country. As per UNAIDS estimates of end 2005, there were 5000 adults with HIV in the country.

The number of new infections that occur every year is estimated to be 140. ART has been made available to HIV-positive people since December 2004. Currently there are 85 patients on ART. There are no facilities to do viral counts and PCR tests for genotyping.

The main challenges faced are capture of HIV positives and treating them with ART. The wide gap between the estimates and actual reported numbers of HIV cases creates a problem for scaling up.

Thailand

Thailand has reported a cumulative number of 1 092 327 HIV infections of which 551 505 have died. The number of people living with HIV is currently 540 822

ART treatment has been expanded since the year 2000. With additional support from the Global Fund, the National HIV/AIDS Task Force set the specific objectives to increase access of the combination of Highly Active Antiretroviral Treatment (HAART) regimen to 88 000 cases under the National Access to ARV Treatment for Patients Living with AIDS Programme (NAPHA) by 2006. Access to treatment for all indicated patients was announced recently by the National Health Security Office. The first-line regimen is a combination of zidovudine (ZDV), lamivudine (3TC) and NVP. Otherwise, short course zidovudine (ZDV) with a single dose nevirapine (NVP) during labour has been implemented nationwide to prevent HIV transmission from mother to child.

A national surveillance system to monitor the occurrence of resistance surveillance strain in HIV-infected patients is being planned. The main objectives shall be: (i) to monitor transmission of HIV drug resistance in naïve populations or threshold surveys, and (ii) to monitor incidence of HIV drug resistance in ARV treated patient cohort study.

There are some laboratories that have the ability to perform drug resistant testing, such as the National Institute of Health. Efforts are being made to obtain accreditation from WHO for undertaking HIV drug resistance genotypic testing.

The country reports brought out the diversity in the HIV epidemic among different countries of the Region. While some countries like India and Thailand faced a very large disease burden, in others it was at a low level. While ART has been introduced in most of the countries, the coverage of eligible population differed from country to country. India and Indonesia have formed National Drug Resistance Committees. None of the countries in the Region has any WHO accredited laboratory for HIV drug

resistance testing. India is in the process of having one of its laboratories accredited. Given the available infrastructure it is evident that it may not be possible for each country to establish accredited drug resistance-laboratories. Intercountry collaboration for drug-resistance testing could be vital for the programme.

5. Laboratory techniques

5.1 WHO HIV Resistance Monitoring Programme (HIVResNet)

The WHO/HIVResNet Global HIV Drug Resistance (HIVDR) Prevention, Surveillance and Monitoring Programme is a collaborative project coordinated by WHO, that has been in operation since 2000. The programme was begun in an effort to gather accurate data on the prevalence and patterns of antiretroviral drug resistance mutations worldwide through the use of a standardized methodology.

The emergence of HIV strains that are resistant to antiretroviral agents (ARVs) is not a new problem, but it has recently received increased attention. Concerns about the observed increase in the transmission of drug-resistant HIV strains have been raised as a consequence of the joint international effort to provide appropriate treatment to millions of persons living with HIV/AIDS in developing countries. If ARVs are not effectively delivered, HIVDR could become widespread, leading to an increase in therapeutic failures and transmission of resistant virus, and a decrease in therapeutic options, and the effectiveness of survival and treatment programmes.

In general, the lack of reliable data and standardized methodologies for sample collection, specimen manipulation and analysis makes interpretation of results very difficult, and the application of this information towards public health action particularly challenging. Currently available drug resistance data are insufficient in quantity and quality to allow an accurate assessment of the extent of the HIVDR problem worldwide. The cost of HIVDR testing poses an additional constraint. In resource-limited countries, HIVDR testing is not generally available, or is too costly to be used in routine treatment monitoring of patients receiving ARV drugs. Therefore, in developing countries, WHO does not recommend that capacity be expanded to provide routine HIVDR testing for individual

clinical monitoring until more basic diagnostic and clinical tests to support HIV diagnosis and treatment have been made widely available.

WHO is approaching universal access by developing an "essential package" for ART scale-up and HIV prevention, including an "essential HIVDR package". With the current effort to provide ARVs to millions of people in developing countries, it is crucial to produce useful and reliable information using a dedicated standardized approach to track HIVDR. To assist ART programmes and to minimize the emergence and transmission of HIVDR strains and the subsequent public health consequences, WHO and other international partners have developed a strategy for "Prevention, Surveillance and Monitoring of HIV Drug Resistance". The intention is that this strategy be implemented in all countries where access to ARVs has been established or where ARV availability is being rapidly scaled up. The national workplan should include:

- Adaptation of country-specific protocols for HIVDR.
- Development of a budget and funding plan.
- Data analysis and interpretation.
- Regular collection, reporting and dissemination of annual reports.
- Evidence-based recommendations for public health action to contain HIVDR.
- Partnerships for all aspects of the work.

5.2 WHO HIV resistance laboratory strategy

HIV drug resistance (HIVDR) can be determined *phenotypically*, in cell culture-based assays, and *genotypically*, by DNA sequence analysis of the HIV reverse transcriptase (RT) and protease coding regions. Genotypic drug resistance testing has now become the method of choice of many laboratories in developed countries and is the technique recommended by WHO for the purpose of HIVDR surveillance and monitoring. Genotyping is performed either by using commercial HIVDR genotyping kits that include reagents, controls and software to generate the results or by using in-house developed or "home-brew" assays. For homebrew assays,

laboratories select their own primers for amplification and sequencing and use generic reagents and software for further sequence analysis.

The WHO/HIVResNet Laboratory Strategy functions to support national, regional, and global HIVDR surveillance and monitoring by the timely provision of accurate genotyping results in a standardized format that meets WHO specifications. The aim of the WHO HIVDR Laboratory Strategy is to outline methods and activities for ensuring:

- (1) The accurate collection, handling, shipment and storage of specimens collected in countries implementing HIVDR Surveillance and Monitoring surveys, and
- (2) The availability of competent HIV genotyping laboratory services at the national, regional and global level.

Principles of the WHO/HIVResNet Laboratory Strategy are:

- (1) HIVDR testing – In the context of HIVDR Surveillance and Monitoring surveys, HIVDR testing must be performed exclusively in WHO-accredited genotyping laboratories.
- (2) HIVResNet Laboratory Network – HIVResNet Laboratory Network is responsible for ensuring the delivery of quality-assured HIV genotyping data at the national, regional and global level. This can be achieved through standardization of drug resistance testing methods in all laboratories accredited by WHO for HIVDR Surveillance and Monitoring surveys.

Membership – Only WHO-accredited genotyping laboratories can become members of the HIVResNet Laboratory Network. The network includes different levels of membership, with different tasks and responsibilities according to rank:

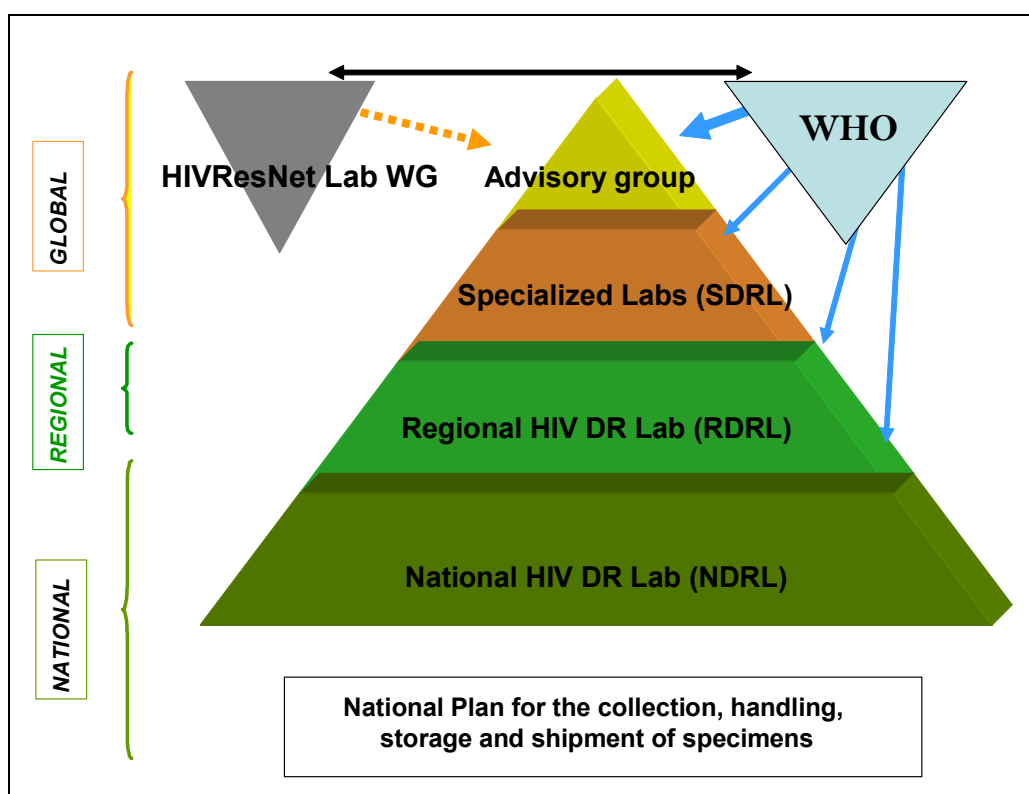
- National HIVDR Laboratories;
- Regional HIVDR Laboratories;
- Specialized HIVDR Laboratories;

The coordination of the Network is a responsibility of WHO in consultation with the Advisory Group, which is composed of representatives of the specialized and regional laboratories.

HIVResNet laboratory network – role and function

The Global HIVResNet Laboratory Network of genotyping laboratories is being developed under the lead of WHO with the objective of delivering accurate genotypic results (at the national, regional and global level) on specimens collected during the monitoring and surveillance surveys. These data can thus be entered into the national, regional and global HIVDR database and be used to target and focus resources on containing and minimizing the spread of drug resistance and to guide decisions of policy-makers on ARV treatment at the national, regional and global level.

Figure 1. Structure of the HIVResNet Laboratory Network



The HIVResNet Laboratory Network aims to ensure high-quality data by:

- Developing laboratory guidelines to describe the complete process of specimen manipulation, including collection, shipment and storage, and HIVDR testing.

- Developing and coordinating an integrated and harmonized quality assurance scheme that operates in all accredited laboratories within the Network.
- Assisting in capacity building and in training of laboratories that are seeking to improve their infrastructure and ability to achieve WHO accreditation.
- Engaging in research projects to identify simpler and more affordable methods for detection of resistance.

The WHO/HIVResNet Laboratory Network consists of three levels of institutions that perform HIVDR genotyping (see figure).

Laboratory exercises

Several introductory presentations were made on the rationale of techniques, collection of material and performance of the tests.

The participants witnessed demonstrations of the various steps of HIV drug resistance genotyping assay(s). They were divided into three batches for the practical sessions in the laboratory. The methodologies for genotyping assays that were discussed included a commercial kit and the 'in-house' method.

Genotyping procedure

The commercially available kit Viroseq™ was used. The steps in HIVDR genotyping procedure are:

- (1) HIV-1 RNA extraction from plasma specimen,
- (2) Reverse transcription (RT) of HIV-1 RNA to complimentary DNA (cDNA),
- (3) Polymerase chain reaction (PCR) amplification of cDNA,
- (4) Amplicon purification and quantification by agarose gel electrophoresis,
- (5) Sequencing reaction and purification of sequencing reaction products,

- (6) setting up of a plate in ABI 3100 sequencer, and,
- (7) Software analysis of sequences and generation of HIV drug resistance genotyping report.

The “in-house” method for RNA extraction and single step RT-PCR followed by nested PCR were also demonstrated. The Kits used for the “in-house” method included QIAamp Viral RNA Mini Kit and RobusT™ I RT-PCR Kit.

The principle of each individual assay steps enumerated above and issues related to trouble shooting were explained to the participants by the facilitators for the workshop.

The following recommendations were made by the participants:

6. Recommendations

6.1 For WHO

The following actions were recommended for WHO:

- (1) WHO should facilitate accreditation of designated national laboratories for undertaking HIV drug resistance studies and participation of these laboratories in HIVResNet.
- (2) WHO should disseminate the surveillance and monitoring protocols for undertaking laboratory-based drug resistance studies to all Member States This will assure accurate interpretation and proper utilization of laboratory results by the National AIDS Programme.

6.2 For Member countries

The following were recommended for Member countries:

- (1) Member countries should accord priority to establish infrastructure for HIV drug resistance monitoring based on their burden of disease.
- (2) Member countries should obtain WHO accreditation of laboratories that are undertaking studies on drug resistance in HIV.

- (3) Member countries should ensure continuous availability of reagents for genotyping to the designated laboratories.

7. Concluding session

WHO's initiative in organizing this meeting was appreciated in the concluding session. The participants also felt that the deliberations shall considerably contribute to developing or improving laboratory support to ART programmes. The meeting ended with a vote of thanks for officials of NARI who conducted this workshop, the facilitators and the international experts.

Annex 1

List of participants

Bangladesh

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Annex 2

Programme

Day 1, 7 August 2006

0830–0930	Registration Inauguration Objectives Mechanism	
0930–1015	Pre-workshop test	
1015–1130	Country reports	Moderator: <i>Dr Rajesh Bhatia</i>
1330–1430	Monitoring of resistance: Discussions	<i>Dr Silvia Bertagnolio</i> <i>Dr Tripathy</i>
1430–1530	Protocol for undertaking monitoring	<i>Dr Silvia Bertagnolio</i>
1530–1700	Overview of laboratory techniques	<i>Dr Sourav Sen</i> <i>Dr Saravana</i>

Day 2, 8 August 2006

0830–0930	Infrastructural requirements for resistance monitoring	<i>Dr Tripathy</i>
0930–1015	Tour of the institute and orientation to laboratory	
1015–1430	Laboratory work in two groups	<i>Prof. Sathien</i> <i>Sukpanichnant</i>
1430–1530	Quality system and need in resistance monitoring	<i>Dr Paranjape</i>
1530–1700	Establishment of EQAS for resistance monitoring	<i>Dr Silvia Bertagnolio</i>

Day 3, 9 August 2006

0830–1230	Laboratory work in two groups	
1330–1430	Review of practical and trouble shooting	
1430–1700	Laboratory work in two groups	

Day 4, 10 August 2006

0830–1130	Laboratory work in two groups	
1130–1230	Bio-safety in laboratories	<i>Dr Paranjape</i>
1330–1700	Laboratory work in two groups	

Day 5, 11 August 2006

0830–0930	Framework for national monitoring of HIV resistance	<i>Dr Silvia Bertagnolio and Dr Paranjape</i>
0930–1130	Discussion on framework in group work	
1130–1230	Sharing of data with national programme and WHO	<i>Dr Paranjape</i>
1330–1430	WHO/HIVResNet	<i>Dr Silvia Bertagnolio</i>
1430–1530	Post-workshop assessment	
1530–1700	Recommendations and valedictory	