Strengthening Laboratory Diagnosis of Multidrug and Extensively Drug-Resistant TB

Report of a regional workshop
Bangkok, Thailand, 22 – 29 September 2009

World Health Organization
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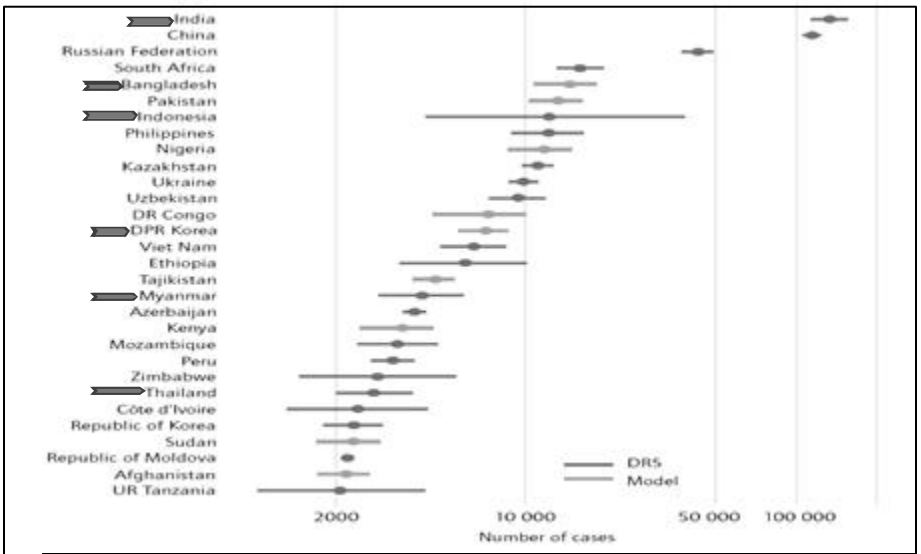
1. Introduction

Over 150 000 TB patients are estimated to develop multidrug-resistant tuberculosis (MDR-TB) every year in the WHO South-East Asia (SEA) Region. This number is based on estimates for multidrug-resistance rates for the Region, which in turn are based on data from a limited number of drug resistance surveys undertaken mainly in six countries in the Region. India alone accounts for more than 85% of MDR-TB cases in the Region and is ranked number one globally with an estimated burden of 131 000 cases occurring annually (Fig.1). The true extent of extensively drug-resistant TB, (XDR-TB) is presently unknown in the Region, given the extremely limited capacity for second-line drug susceptibility testing, though more than one case has been reported from India. Bangladesh, Indonesia, DPR Korea and Thailand are the other countries besides India in the Region that have been classified under the 30 countries having the highest number of estimated MDR-TB globally.

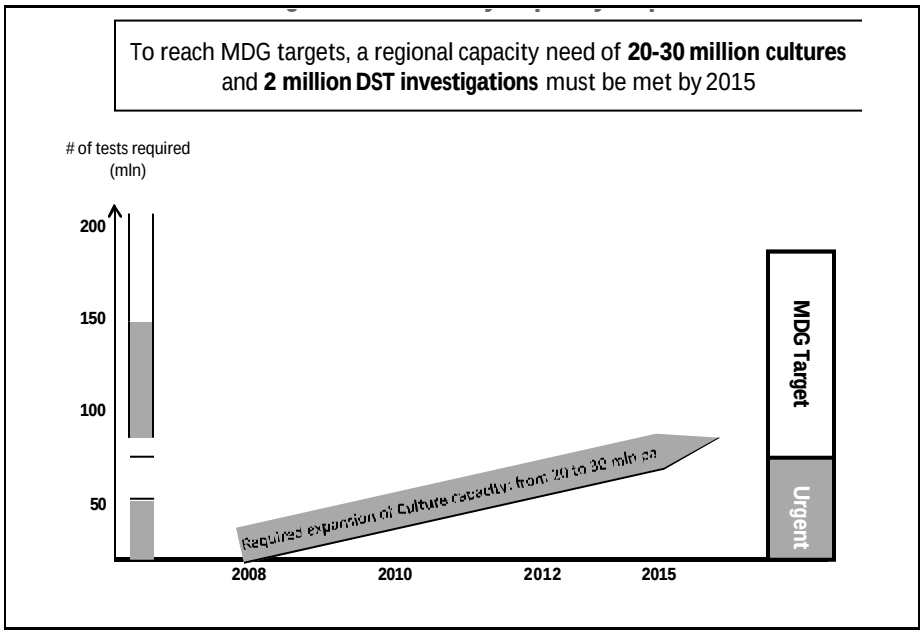
Figure 1: ***Countries with the highest numbers of estimated MDR-TB cases, 2007***



Horizontal lines denote 95% confidence intervals. The source of estimates is drug resistance surveillance or surveys (DRS, in red) or modelling (in grey)

One of the prerequisites for addressing MDR-TB and XDR-TB is developing the capacity of national laboratory networks to detect drug-resistant TB through quality-assured culture and drug-susceptibility testing, and deployment of newer diagnostics now available, as well as undertaking regular drug resistance surveillance to determine trends in the MDR/XDR-TB. Figure 2 shows the quantum of regional capacity required for culture and drug-susceptibility testing (DST) to meet the Millennium Development Goals (MDG) by 2015.

Figure 2: ***Required expansion of regional laboratory capacity***



While national reference laboratories (NRLs) in all Member states (with the exception of DPR Korea, Maldives and Timor-Leste) now have the capacity for mycobacterial culture, this capacity is quite limited. National reference laboratories in Bangladesh, Indonesia, Myanmar and Sri Lanka have recently been quality assured for culture and first-line drug susceptibility testing, while in Nepal, these facilities are provided through a nongovernmental (NGO) run laboratory, pending the accreditation of the newly- established national reference laboratory. National reference laboratories in India and Thailand are the only two laboratories in the Region currently undertaking for second-line anti-TB drugs to detect XDR-TB.

This workshop was organized in order to meet the felt need to improve the technical and managerial capacity of senior TB laboratory managers to ensure quality-assured microscopy, culture and drug susceptibility testing, as well as to commence using newer diagnostics for detection of drug resistance, both for the purposes of expanding diagnosis of M/XDR-TB within countries and for undertaking regular drug resistance surveillance to monitor trends in TB drug resistance.

2. Objectives of the workshop

The objectives were to:

(1) Review the MDR/XDR TB situation in Member States and the capacity of national laboratories to undertake diagnosis of MDR/XDR-TB;

(2) Enhancing skills for laboratory techniques for diagnosis of M/XDR-TB; and

(3) Identify country-specific next steps for laboratory scale-up for diagnosis of M/XDR-TB.

3. Inaugural session

The programme commenced with the introduction of the workshop objectives and the role of laboratory in the management of MDR/XDR TB by the Head, Supranational Reference Laboratory, Bangkok, Thailand, who was the local coordinator. This was followed by the address of the Director, TB Cluster, Department of Disease Control, Ministry of Public Health, Thailand. In his address, the Director highlighted the problem of MDR-TB in the Region along with the importance of the role of laboratory capacity as a critical factor for scaling up MDR treatment services in the Region.

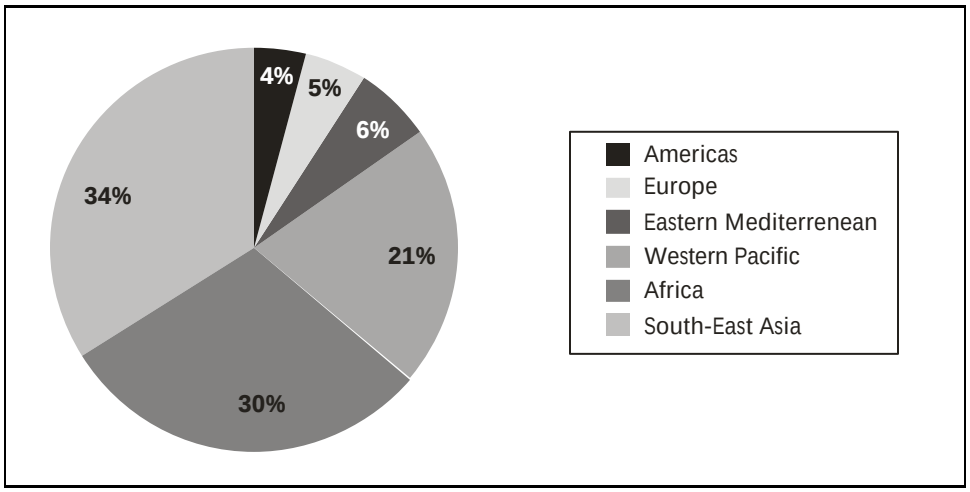
This was followed by comments from all facilitators on the importance of laboratory capacity-building and the need for total commitment both in respect of the political and technical aspects of management of MDR/XDR TB, as the Region had five of the 30 high-burden countries with TB and two countries with highest rates of estimated MDR-TB in the world, namely India and Indonesia.

4. Technical Sessions

4.1 Laboratory capacity to manage MDR/XDR TB in the Region

Tuberculosis is a major public health problem with over nine million cases per year and 1.7 million deaths. It is predominantly a disease of poverty and resource-poor, high-burden countries. Despite concerted global efforts for the control of TB, it still continues to be a problem. It is now compounded with problems of HIV co-infection and drug resistance. Drug resistance is a natural, and biologically unstoppable phenomenon. All antimicrobials have the potential to select drug-resistant subpopulations and are generally slowly reversible or irreversible. The global prevalence of multidrug-resistant TB is estimated to be 51 100 cases and that of XDR-TB approximately 50 000 cases. In order to diagnose and treat these XDR-TB cases, sufficient laboratory capacity is needed. However, it is lacking in many countries that are estimated to have the highest burden of MDR/XDR TB. Laboratory capacity to manage MDR/XDR TB is a complex and a critical need that has huge challenges. The Region faces the largest problem, accounting for one third of the global problem (Fig. 3) and >90% cases in five countries, Bangladesh, India, Indonesia, Myanmar and Thailand.

Figure 3: ***Estimated incidence of all forms of TB by WHO Region, 2008***



Most countries of the Region have limited laboratory capacity to diagnose MDR/XDR- TB, due to constraints in developing infrastructure, HRD issues and limited access to modern technologies for rapid diagnosis of MDR-TB. Most laboratories do not have the requisite equipment, and bio-safety levels as recommended for handling *M. tuberculosis* isolates. Most of them also lack adequate numbers of skilled laboratory personnel at all levels within the national laboratory networks. In order to strengthen laboratory capacity, needs assessment should be carried out of the existing capacity and gaps, and detailed plans for building laboratory capacity should be developed with the assistance of laboratory experts. The introduction of new technologies for conducting rapid diagnostics for the detection of all forms of TB, including MDR-TB, is critical to ensure faster detection, as well as to reduce the workload on laboratories.

Plans should include a clear definition of the functions at each level of laboratories, the number of facilities required, number of personnel and skills, and training requirements. Plans also need to take into account the mechanisms for quality assurance, and technology transfer for deployment of newer diagnostics. Guidelines for all laboratory procedures including bio-safety, infection control, and collaboration with supranational reference laboratories for training, quality assurance and supervision need to be established.

Laboratory infrastructure development is a complex and technically demanding exercise involving huge capital investment. Hence a high level of political commitment, as well as support from all partners involved in the country's disease control programme are needed. Better coordination between partners and donors is required in order that the resources are optimally utilized and for countries to maximally benefit from the support provided.

Countries should build their laboratory capacity rapidly and should be able to offer laboratory services to the entire TB community of the country, through an efficient system of laboratory network and transport of specimens from all parts of the country. The take-home message is that ***"TB laboratories cost a lot but the absence of these facilities cost more".***

4.2 Laboratory safety

M. tuberculosis infections are a proven hazard to laboratory personnel as well as others who may be exposed to infectious aerosols in the laboratory. Careful analysis of data for incidence of infection and TB among laboratory technicians has shown that they carry a higher risk of TB compared to other health-care workers.

Bio-safety issues related to laboratory are addressed in many documents. Some countries in the Region have developed their national bio-safety guidelines.

Guidelines for administrative controls for TB laboratory safety relate to exposure to laboratory-generated aerosols (Fig. 2). Accidental needle-stick injury is also a recognized hazard. Administrative controls include development of policies and procedures for mycobacteriology laboratories, including policies for use of WHO- recommended procedures and methods, Standard Operating Procedures (SOPs) for all processes, waste disposal, safety strategies for prevention of aerosols and spill avoidance, spill response plan and recommended management of a spill. Chemical safety with regards to the use of alcohols, avoidance of phenol toxicity and handling of acids, etc. requires attention from the bio-safety aspect. This should also extend to specimen collection, transport, shipment and disposal. It is recommended that each laboratory should develop or adopt a bio-safety or operations manual as per the country's laboratory policy.

Facilities such as secondary barriers including elbow-or foot-operated handwashing facilities, separation of the laboratory work area from public access, and availability of a decontamination facility (e.g. autoclave) should be established preferably in separate buildings or modules to isolate the laboratory.

Disinfection by discarding specimens in mycobactericidal disinfectants such as 5% phenol or 2% chlorine containing disinfectants is preferred over mycobacteriostatic disinfectants such as biguanides or quaternary ammonium compounds.

Bio-safety levels 1 to 4 (BSL1 or BSL IV) for secondary barriers are based on the layout of the laboratory, and safe equipment and laboratory practices. Facilities such as controlled access to the laboratory, foot-or

elbow-operated handwashing sinks; autoclaves for waste decontamination should be provided near or in the facility itself with horizontal double-door autoclaves, wherever possible, and if funds permit. Appropriate maintenance of (BSCs) is mandatory. For negative pressure facilities, airflow of at least 12 ACH with negative air pressure of 15-25 Pascal is required. Replacement of pre-filters for BSC should be done at least once in six months and high efficiency particulate air (HEPA) filters of BSC and /or negative pressure unit should be done at least once a year.

Personnel protection should be ensured through training of laboratory personnel in infection control in laboratory and monitoring of equipment. Personnel must confirm that air flow is unidirectional throughout the facility and that negative air-pressure gradients are maintained. Safe practices such as wearing of laboratory coats or gowns over street clothes, and wearing gloves overlapping the sleeves of the gown and removing these when leaving the laboratory, should be ensured.

The checklists that should be included in the monitoring and evaluation guidelines are: (i) availability of recent national TB laboratory biosafety guidelines; (ii) integration of national TB laboratory biosafety guidelines with national bio-safety guidelines, (iii) inclusion of laboratory bio-safety aspects, including "illness surveillance" in supervision checklists for national, regional and peripheral-level laboratories, (iv) training of all laboratory staff in biosafety; and (v) annual refresher training of all laboratory staff in bio-safety.

4.3 Developing a comprehensive quality assurance system

Quality assurance with regard to tuberculosis bacteriology is a system designed to continuously improve the reliability, efficiency and use of tuberculosis laboratory services. The purpose of a quality assurance programme is to improve the efficiency and reliability of laboratory services. In order to achieve the required technical quality in laboratory diagnosis, a continuous system of quality assurance needs to be established. The components of a quality assurance programme are: quality control; quality improvement; and proficiency testing.

Quality control is a process of effective and systematic monitoring of the performance of benchwork in the tuberculosis laboratory against

established limits of acceptable test performance. It ensures that the information generated by the laboratory is accurate, reliable and reproducible, and serves as a mechanism by which tuberculosis laboratories can validate the competency of their diagnostic services.

Quality improvement is a process by which the components of tuberculosis laboratory services are analysed continuously to improve their reliability, efficiency and utilization. Proficiency testing is part of the programme of external quality assurance and is ensured through a network of supervising laboratories like the WHO supranational reference laboratories or professional organizations like the College of American Pathologists (CAP) and the International Standards Organization (ISO), etc.

Quality control encompasses all aspects of laboratory management, viz. physical structure, procurement of quality equipment, check and maintenance of all equipments and that all procedures are performed in the laboratory from specimen receipt to reporting of results. All chemicals, reagents and kits that are used for processing specimens are subject to quality checks. Diagnostic procedures commencing from decontamination, smear microscopy, culture and drug susceptibility testing, whether performed manually or using automated systems are subject to quality control.

Quality improvement involves data collection, data analysis and creative problem-solving that are the key components of this process. It involves continuous monitoring and identification of defects, followed by remedial action to prevent recurrence of problems. Often, problem-solving can be done efficiently only during on-site supervisory visits. These visits are the quickest and most effective form of quality improvement because of the personal contact and because they permit on-the-spot corrective action. *Supervision should always be done by more experienced laboratory personnel.*

External Quality Assurance in line with WHO standards refers to a system that retrospectively and objectively compares results from different laboratories by means of programmes organized by the Supranational Reference Laboratory or the National Reference Laboratory to check the quality of drug susceptibility testing performed by testing laboratories. It is a dynamic process that is required to be repeated every six months or annually. Proficiency testing is a programme designed to allow participating laboratories to assess their capabilities by comparing their results with those

obtained in higher-level laboratories. For this purpose, material for testing is prepared by the central or reference laboratory and distributed to lower-level laboratories. The recipients perform the necessary procedures and report their results to the central or reference laboratory, which can then assess proficiency. Detection of deficiencies through this indirect system will then determine the need for quality improvement. Participation in proficiency-testing programmes may be compulsory, as in hierarchical laboratory organizations, where the central or reference laboratory is responsible for those at the lower levels. It may otherwise be voluntary, where a specific quality control laboratory (QCL) of the public health laboratory service provides a variety of material to any interested laboratory. Each laboratory has a unique identification code known only to itself and the QCL. The QCL sends out collective reports that enable each of the participating laboratories to compare its proficiency with that of the others. Irrespective of whether proficiency-testing programmes are compulsory or voluntary, a minimum requirement is laid down by which there is a proficiency panel testing for drug susceptibility testing (DST) of first line DST(SHRE) and second line drugs (K, AK, CAP, OF), wherever available.

External Quality Assurance for Culture is more difficult and is possible only to a limited extent, and is mainly done during on-site evaluation by careful analysis of data in the laboratory culture registers. Panels of freeze-dried specimens or aseptically prepared artificial specimens with known counts of *M.tuberculosis* organisms marked for a specific genotype or drug resistance pattern can be used to evaluate culture capacity, including identification and cross-contamination. This does not reflect the reality of real time performance. A quality assistance programme (QAP) can serve as a learning exercise, enabling the recognition and identification of problem areas that might otherwise have been overlooked. *A good quality assurance programme will enhance the credibility of the laboratory.*

4.4 Use of culture: diagnostic efficacy, role and policy - methods and technical aspects

The laboratory tools for diagnosis of TB include:

- Demonstration of mycobacterium tuberculosis in clinical specimens.
- Smear microscopy (Ziehl-Neelsen, fluorescent examination of direct and concentrated specimen) and newer versions eg,:

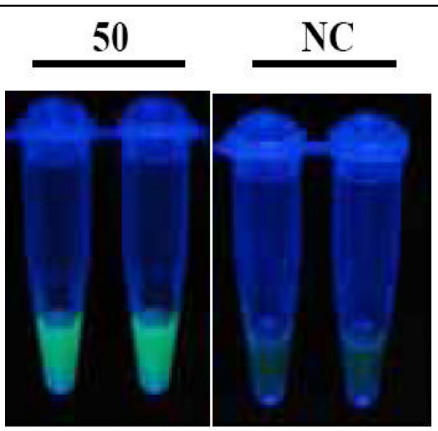
battery-operated light-emitting diode (LED) microscope with both bright field and FM attachment.

- Culture examination (conventional, rapid automated systems)
  - Egg-based, agar-based media.
  - BACTEC, Mycobacteria Growth Indicator Tube (MGIT), BacT Alert
- DNA/RNA Nucleic Acid Amplification Tests (NAAT) – newer methods eg,: X-pert TB, loop amplified mediated amplification assay (LAMP) (rapid detection of *M.tuberculosis* and rifampicin resistance).
- Detection of *M.tuberculosis* constituents
- Immunological methods
  - Detection of antibodies to species-specific/disease-specific *M.tuberculosis* antigens (serology).
  - Interferon-gamma (IFN) gamma release assays (IGRA).
  - Quantiferon GOLD assay.

Figure 3: ***Some examples of new technologies***

Battery–operated LED microscope

X-pert TB test



LAMP test

Any culture method that would be reliable and useful for use in a national TB control programme requires the following characteristics:

- Technical accuracy/reproducibility (sensitivity, specificity, positive predictive value, negative predictive value).
- convenient for case management (turnaround time - TAT).
- accessibility to the entire population.
- operational feasibility in local settings: technical simplicity, facility, equipment, trained motivated staff, quality assurance.
- cost-effectiveness for patient care.

Culture facilities in the laboratory network must not be set up at the expense of smear microscopy services. There should be a good referral system for transport of sputum specimens in a safe and rapid manner. The culture laboratory should preferably be centralized with access to all patients. Culture examination is more sensitive and is essential for drug sensitivity testing for identification of drug-resistant TB. It is also required to diagnose smear-negative cases, paediatric TB, extrapulmonary TB and TB-HIV co-infected patients.

The disadvantages include a long TAT (conventional solid culture), requirement of equipment, complex technical skills and trained man power. The culture efficiency is greatly affected by quality, quantity and freshness of sputum specimen, decontamination procedure (centrifugation, exposure time to decontaminant), and culture medium and growth conditions. The culture techniques from decontamination to culture reading were explained in detail with the advantages and disadvantages of each decontaminant, method of decontamination and different media used. Processing specimens by the simple method and with the centrifugation method was discussed and the use of each method in appropriate situations was emphasized.

This was followed by a discussion on incubation conditions for optimal growth of *M.tuberculosis* in various culture media (advantages and disadvantages of solid and liquid media). The standard recording and reporting formats for mycobacterial culture examination, along with the identification of mycoabcterial species by conventional biochemical tests and quality issues related to culture were discussed in detail.

4.5 Practical exercise on culture technique

During this session, the participants were divided into four groups. Each group worked with the assigned facilitator and observed the procedure of specimen decontamination and inoculation being performed by technologists of the SRL. They were subsequently asked to process two specimens each under the supervision of the facilitator and SRL technologist. All queries related to culture examination posed by the participants were addressed by facilitators.

4.6 Drug-sensitivity testing

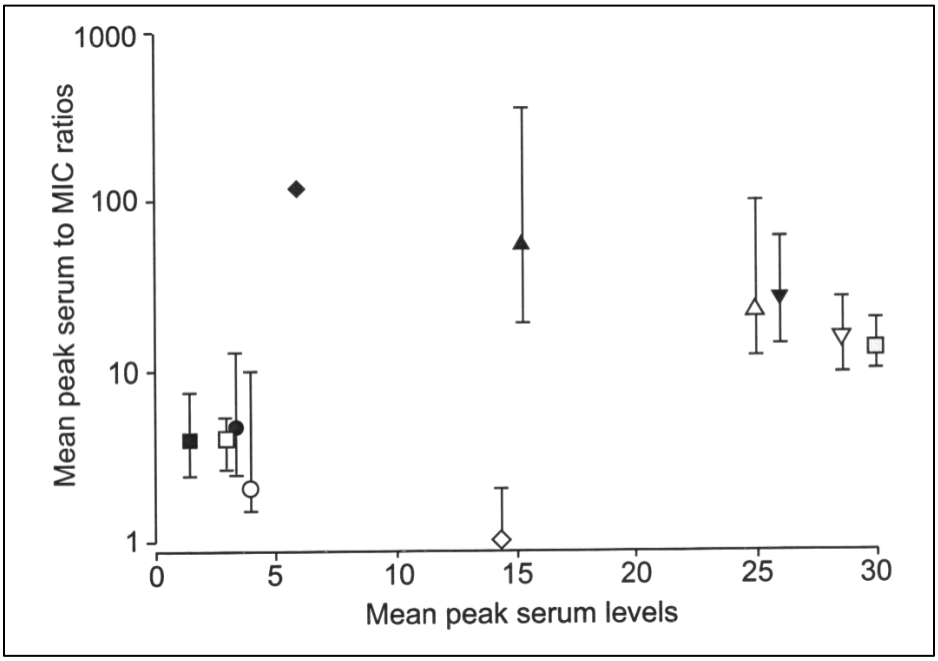
This was discussed in great detail with an initial introduction to the basics of development of drug resistance by *M.tuberculosis* in wild strains and in patients who have taken irregular drug therapy. Definitions of resistance along with the mode of acquiring drug resistance were discussed in detail. The techniques of drug susceptibility testing (DST) explaining the different methods, advantages and disadvantages of using them, the role of DST in various settings, the technical skills required to set up DST in laboratories, the bio-safety issues, the quality of drug powders used for testing and the reliability and accuracy of DST were presented.

The calibration step of DST is critical for determining the criterion for resistance for clinically-relevant strains, by assessing the level of susceptibility of presumably susceptible strains, determination of level of susceptibility at which treatment response changes, which is done by a comparison of susceptibility by a sample of strains from never-treated patients with that of a sample of strains from patients who have been treated with the drug for at least five/six months. If the frequency of distribution of probably susceptible (PS) and probably resistant (PR) strains has minimal overlap and the gap between PS and PR is largest when cumulative percentages are plotted (for eg INH and RIF), the laboratory definition of resistance correlates very well with clinical response to the drug. However, except for these two drugs, most other anti-TB drugs do not have such a sharp demarcation, and hence have less reliability.

The definition of resistance is also affected by the stability of the drug in the medium, the therapeutic index and technical expertise for preparation of drug media, bacterial suspension and inoculum size. The

resistance-predictive value of drug susceptibility test results was explained by the frequency of probably susceptible strains/probably resistant strains. Inhibited at different drug concentrations and the cumulative distribution of susceptibility to INH and to RMP (tests done in L-J medium using the absolute concentration method and proportion method), was explained. The comparison of critical concentrations of ethambutol between two different laboratories (tests done in L-J medium using the absolute concentration method) and cycloserine susceptibility patterns of probably susceptible and probably resistant clinical isolates of *M. tuberculosis* were explained.

Figure 4: ***Approximate mean peak serum to minimal inhibitory concentration ratios of anti-TB drugs***



The proportion method was explained in depth as it is one of the commonly used methods for drug resistance surveys. The reliability of DST results to better predict outcome of treatment was discussed. The importance of proficiency testing and EQA of drug media were highlighted with data from the 14th round of proficiency testing results being illustrated as an example for

concordance and agreement for resistance testing of various drugs among the SRLs participating in the exercise as illustrated below.

Agreement of DST results to Isoniazid and Rifampicin of the 51 National (or Regional) Reference Laboratories (WHO Fourth Report of Global Drug Resistance, 2008).

Table 1: *Concordance among National Reference Laboratories for DST*

Percentage Agreement	Isoniazid	Rifampicin
100	38 (77.6)	37 (72.5)
95-99	6 (12.2)	9 (17.6)
90-94	4 (8.2)	5 (9.8)
<90	1 (2.0)	0
Total number of laboratories	49 (100.0)	51 (100.0)

4.7 Practical exercise on quality control

Participants were asked to make a macroscopic evaluation of the media (good, bad, overcooked, insufficient heating, contaminated). They were then given good and bad media for inoculation using *M.fortuitum* (as a proxy marker for *M.tuberculosis*), so that this could be read on the penultimate day to differentiate the effect of quality of media on growth.

4.8 Practical exercise on drug media preparation

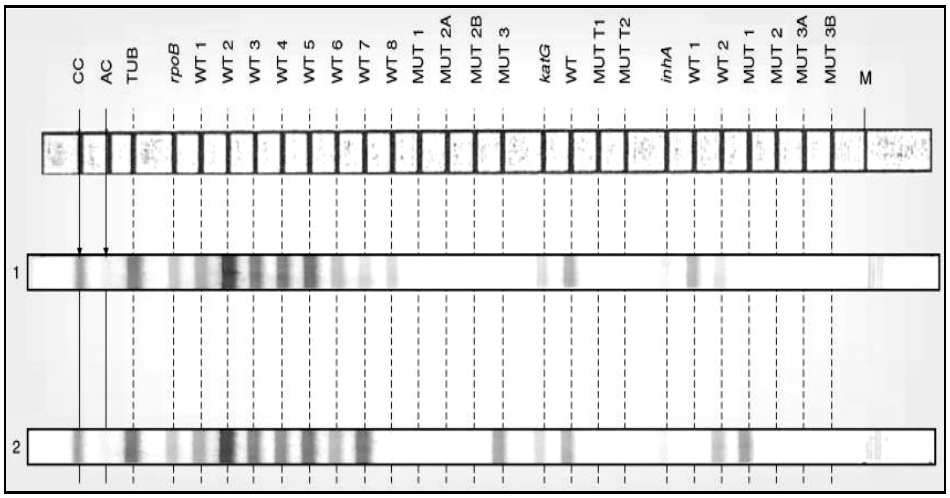
The four groups were given a demonstration on drug media preparation by the SRL staff. This was followed by discussions with the facilitators on the various factors that could affect drug media preparation, which in turn, would influence DST results.

Factors such as the importance of volumes (that are measurable) to be chosen for preparing solutions; close monitoring of inspissation temperatures, use of pure drug powder only, and potency calculation for each drug before weighing the drug powder were emphasized.

Rapid detection of drug resistance by Line Probe Assay using the Hains MDRTB plus – a commercial molecular test

The lecture commenced with the introduction to the technology of Line-probe assays, a family of novel DNA strip-based tests that use PCR and reverse hybridization methods for the rapid detection of mutations associated with drug resistance. They have very high sensitivity and specificity when used on culture isolates. Two assays: *Inno-LiPA Rif* and *Genotype MDR-Plus* are available as commercials kits. The main advantage of using the genotype MDR TB plus is that it has been modified to be used directly on sputum specimens and possesses improved sensitivity to detect INH resistance with two genes (*kat G* and *inhA*) being used in this assay. This can theoretically achieve an excellent TAT of eight hours by which time you can detect whether the patient is having an *M.tuberculosis* isolate with MDR-TB resistance or not.

Figure 5: ***Genotype MDR TB plus test***



In practice this test has revolutionized the diagnosis of MDR by making available on a commercial platform a test that can be used by NTPs to diagnose in 48-72 hours based on the time required to transport sputum to the testing laboratory. The principle behind the Line probe assay was explained as shown in the figure below. The genotype MDR- TB plus assay when performed as per manufacturer's instructions will detect the *rpoB* gene for rifampicin resistance, *Kat G* and *inhA* genes for INH resistance and genes for identification of *M.tuberculosis* complex.

The entire procedure starts with DNA extraction, PCR amplification, hybridization, colorimetric detection, evaluation and interpretation of results.

The most important aspects that are required for performing molecular tests include a clean environment, pipettes, sterile plastiware, training and mechanisms to transport specimen within 48 hours if required in cold chain under tropical conditions.

When used under appropriate conditions, these tests are most useful for rapid diagnosis of MDR-TB.

Rapid culture methods

The new guidance document published by WHO in 2007 on the use of liquid culture in low-and middle-income settings was presented. The basic principle of rapid culture for *M.tuberculosis* is the use of liquid media to reduce the time required for growth of detectable *M.tuberculosis* in culture. With major advances since 1980 with the introduction of BACTEC 460 TB system, there are a number of newer automated systems like the MGIT 960, manual MGITand BacT Alert, which are being used for rapid culture and DST.

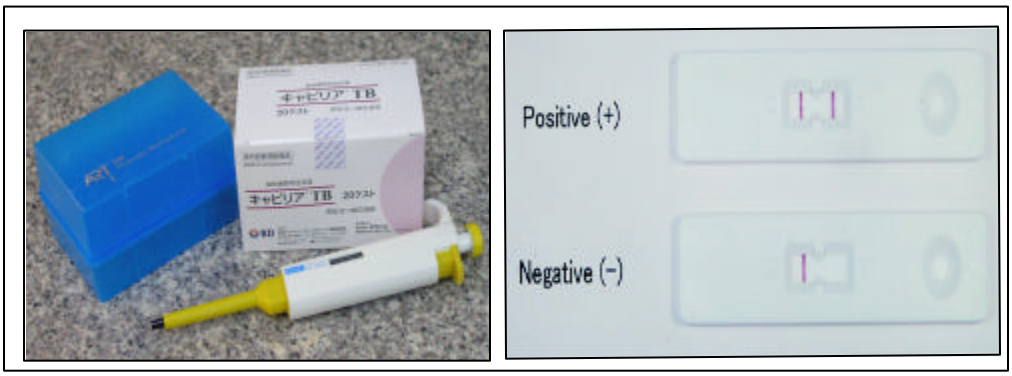
The main indication for using liquid culture systems in resource-limited settings is to identify MDR-TB. The specimens have to be transported fresh in cool box (temperature should not exceed 15-20$^{?}$C), with no preservatives, decontaminated using a milder agent, and the box should have an appropriate biosafe environment for processing them. Due to the high sensitivity of the liquid culture (10-100 bacilli), it is prone to contamination and may not be appropriate in many settings.

The MGIT 960 system is an automated liquid culture system that is capable of incubating 960 tubes simultaneously; tubes are made of plastic with fluorescent sensors and require continuous monitoring. The MGIT 960 requires continuous electricity supply. The procedures for culture inoculation and reading of cultures using MGIT were discussed and compared with conventional methods. This was followed by an interactive discussion with participants on the advantages and disadvantages of using

liquid and solid culture methods and appropriate settings under which a liquid culture system could be an advantage for the NTP.

This was then followed by a description of speciation of culture positives by the new immunochromatographic test, the *Capillia TB*. The test is a simple lateral flow technique that has antibodies to the species-specific MBP64 secretory antigen that is present in *M.tb* strains. The test does not require any sample processing or instruments. The test kit and results are illustrated below.

Figure 6: ***Capilia TB test***



4.9 Practical exercise on drug-susceptibility testing

The four groups of participants were first given a demonstration on setting up of a DST from preparation of drug suspension to the final inoculation onto drug containing media. The preparation of various dilutions was demonstrated using the pipette method. This was followed by each participant inoculating two isolates as demonstrated. A detailed discussion took place with facilitators on procedures, technical skills and quality issues related to DST.

4.10 Practical exercise on DST reading (two sessions)

The participants were given an exercise, in which results of DST reading of 30 strains were recorded, and they were asked to classify them as sensitive or resistant based on the proportion method calculation. After this exercise, the results on readings, number of colonies in each dilution and possible explanations for each of the findings were discussed with facilitators.

The second session was conducted in the laboratory, where the participants read four sets of DST; they were asked to comment on the quality of media, controls and inoculum size on interpretation. Another four sets were given to them for taking the actual readings and interpreting them as resistant or sensitive. This was again followed by a discussion with facilitators on all aspects of DST and the possible reasons and explanations for observed features during the course of DST readings made by participants.

5. Group discussion on laboratory plans, by country

This session was initiated with an introduction to participants on how to prepare a country-level laboratory network plan. Participants of each of the countries were then asked to review the previous plan prepared by the team in 2007 and its current progress. This was followed by a discussion on the constraints and reasons for delays in the implementation of plans. The main issues were related to inadequate funding for infrastructure development, need for technical assistance for procurement and planning, HRD and HR in the context of setting up national reference laboratories and regional reference laboratories.

The participants were then asked to fill in the current status of the laboratory network and areas in which they would require technical assistance from the Regional Office and the supra-national reference laboratories.

Status of national laboratory networks in the Region

All Member States had a quality-assured smear microscopy laboratory network in place with functional EQAP services.

At least one national reference laboratory was functional in every Member State except in Bhutan, Maldives and DPR Korea.

Equipments for solid culture and DST were in place and functional in most countries except in DPR Korea (ongoing), Bhutan (ongoing), Maldives (ongoing) and Timor-Leste (not planned).

National reference laboratories of India, Thailand and Myanmar have been quality-assured regularly and have good links with the SRL.

Indonesia has five regional reference laboratories that have been proficiency-tested through the Supranational Reference Laboratory and the Institute for Veterinary and Medical Sciences, Adelaide, Australia. Indonesia is in the process of identifying its national reference laboratory.

Drug resistance survey data are available from India, Indonesia, Myanmar, Nepal and Thailand.

DPR Korea will shortly be initiating drug resistance survey with assistance from Supranational Reference Laboratory (SRL) China, Hong Kong Special Administrative Region (Hong Kong SAR); its linkage with this SRL needs to be formalized.

In addition, the NRL in DPR Korea is being supported by Stanford University, United States of America, for infrastructure upgradation and technical assistance for TB culture and DST in close collaboration with SRL "Hong Kong SAR".

The NRL in Sri Lanka is functional and has recently upgraded its facility. It will be accredited through the SRL-Tuberculosis Research Centre (TRC) Chennai, India by June-July 2010 to which it is linked.

Bhutan has identified an NRL and is in the process of its accreditation with the SRL, Bangkok, Thailand.

Maldives will develop an NRL at the Indira Gandhi Memorial Hospital, Male and will link it to SRL-TRC, Chennai, India for any services that it may require in the interim.

Nepal is in the process of proficiency-testing for the NRL and is supported by the GENTUP laboratory (supported by SRL Gauting, Germany), an NGO that is responsible for DST services for the MDR treatment programme.

Bangladesh is being supported by both the SRLs at Bangkok, Thailand and at Antwerp, Belgium, and is in the process of developing its network of quality-assured laboratories.

Timor-Leste does not have an NRL and is being supported by Institute of Medical and Veterinary Science (IVMS), Adelaide, Australia.

6. Conclusions and next steps

Based on the status of the laboratory networks in the Region and the plans for expanding the scope of diagnostics and the coverage of laboratory facilities capable of offering these services in the respective countries, the following actions were proposed:

- Most Member States have plans for expansion of culture and DST services for the diagnosis of MDR /XDR TB
- India and Myanmar have approved plans for uptake of newer tools (Line Probe Assay and Liquid culture and DST) for fast-tracking of MDR-TB diagnosis, and are in the process of introduction of these services into their national programmes.
- Most countries have linked the diagnostics to treatment services and secured funding for laboratory expansion and drug procurement through Green Line Committee, Global Fund, 3 Disease Fund, UNITAID and USAID with the exception of Maldives where the drugs are being procured through national funds.
- Standard operating procedures, manuals for laboratory procedures and biosafe practices are in place in India, Indonesia, Myanmar, Sri Lanka and Thailand. The other countries are in the process of preparing them.
- Most countries are in the process of developing their infection control plans for health-care settings, including laboratories.
- All countries are in the process of expanding laboratory services by establishing additional facilities based on local epidemiology and needs assessment for inclusion of newer tools for rapid diagnosis of all forms of TB including MDR/XDR TB.
- Training and skill development of laboratory personnel is required for establishing basic culture and DST services in Bangladesh, Bhutan, DPR Korea, Maldives and Timor-Leste.

India, Indonesia, Myanmar, Nepal Sri Lanka and Thailand need additional skill development and training for adapting newer diagnostic tools.

- In order to move forward in establishing and expanding laboratory services in the 11 Member States of the Region, there is a need for technical assistance and support for situation analysis, developing laboratory plans, resource mobilization and for implementation of such services, which have been classified under the following broad areas:

The main areas for which technical assistance was requested were:

(1) Technical assistance for developing national laboratory plans;

(2) Training – especially at national level (to have more participants);

(3) Assistance for introduction of newer technologies; and

(4) Closer linkages with SRLs and other NRLs.

Annex

List of participants

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Over 150,000 TB patients are estimated to develop multi-drug resistance every year in the South-East Asia Region. Developing the capacity of national laboratory networks to detect drug-resistant TB through quality-assured culture and drug susceptibility testing and through deploying the newer diagnostics now available is a critical pre-requisite for managing as well as monitoring trends in drug-resistant TB.

The workshop on Strengthening Laboratory Diagnosis of Multidrug and Extensively Drug-Resistant TB was organized in order to meet the felt need to improve the technical and managerial capacity of senior TB laboratory personnel. The workshop emphasized on quality-assured microscopy, culture and drug susceptibility testing, as well as to commence using newer diagnostics for the detection of drug resistance both for the purposes of expanding diagnosis of M/XDR-TB within countries and for undertaking regular drug resistance surveillance to monitor trends in TB drug resistance.

The workshop was very interactive, with a mix of presentations, practical demonstrations and hands-on practice sessions. In consultation with the facilitators, participants reviewed the current laboratory capacity in their respective countries and identified the next steps for follow-up at country level.

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