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Regional Workshop on Strengthening Laboratory Services for TB Control

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Contents

Abbreviations

BACTEC	Rapid TB Mycobacterial culture method
BACTEC MGIT 960	Mycobacterial growth indicator tube (liquid culture media)
BACTEC 460 TB	Rapid Mycobacterial culture media
BPS	Basic package of services
DOTS	The internationally recommended strategy for TB control
DRS	Drug resistance surveillance or survey
DST	Drug susceptibility testing
EQA	External quality assurance
IPT	Isoniazid preventive therapy
ISTC	International Standards for Tuberculosis Care
L-J Medium	Lowenstein-Jensen medium
LQAS	Lot quality assurance scheme
MDR-TB	Multidrug-resistant tuberculosis (resistance to at least isoniazid and rifampicin)
NGOs	Nongovernmental organizations
NRL	National reference laboratory
NTP	National TB Control Programme
QA	Quality Assurance
SEA	South-East Asia
SLD	Second-line drugs
SNRLs	Supra national reference laboratories

SOP	Standard operating procedure
TB	Tuberculosis
TB/HIV	Tuberculosis / Human immunodeficiency virus
The Union	The International Union Against Tuberculosis and Lung Diseases
WHO	World Health Organization
XDR-TB	Extensively drug-resistant tuberculosis

1. Introduction

In addition to effectively implementing the DOTS strategy for the diagnosis and treatment of TB cases, patients with multidrug resistance also need to be effectively diagnosed and managed as envisaged under the new Stop TB strategy. Addressing MDR-TB and potentially extensively drug resistant TB (XDR-TB) primarily requires developing the capacity of national laboratory networks to diagnose drug resistant TB through quality assured culture and drug susceptibility testing (DST). The regional workshop on strengthening laboratory services for TB control was organized to meet a felt need to improve the technical and managerial skills of senior TB laboratory managers to ensure quality assured culture and DST.

The week-long course imparted the necessary skills required to establish, as envisaged in the regional and country plans for TB control, national laboratory networks for quality assured smear microscopy and, in addition, quality assured culture and DST. The timing was opportune in the light of on-going efforts in countries to establish culture and DST facilities.

The workshop was attended by 21 senior TB laboratory managers from nine Member countries of the SEA Region and one from the Western Pacific Region. The facilitators for the workshop were from the TB Central Reference Laboratory and International Training Centre in Thailand, the Union, WHO/SEARO, and WHO/HQ.

2. Objectives

The objectives of the workshop were to;

(1) review the TB situation in the Region with particular focus on MDR/XDR TB status and the capacity of national laboratories for undertaking quality assured diagnosis and surveillance of MDR/XDR-TB;

(2) update knowledge on approaches for improving the organization, management and monitoring of laboratory services, including infection control measures;

(3) enhance skills for quality-assured culture and drug susceptibility testing (DST) techniques, and

(4) develop country-specific steps for strengthening laboratory support to National TB Control Programmes for quality-assured diagnosis of TB and surveillance of MDR-TB.

3. Inaugural Session

Dr Tanaphan Fongsiri, Chief of International Training Centre, Bangkok, who chaired the session, welcomed the participants and WHO staff and briefly introduced the objectives of the workshop. Dr Pavongsak Rienttrairat, Senior Medical Officer, TB Cluster also welcomed the participants.

The message of Dr Samlee Plianbangchang, Regional Director, WHO South-East Asia Region, was delivered by Dr Charles Delacollette, Acting WHO Representative, Thailand. The Regional Director stressed that the success of the DOTS Strategy alone was not sufficient to achieve the TB-related targets under the Millennium Development Goals. He mentioned that this workshop was being held to support capacity building to undertake the additional interventions under the new TB Strategy, which aims at detecting all forms of tuberculosis, including drug-resistant TB. He said that this required building capacity for culture and drug-sensitivity testing in the diagnostic package for TB, and offering facilities for the management of drug-resistant TB routinely, by national tuberculosis programmes, at all appropriate levels within national health systems.

In her address, Dr Daranee Wiriyakijja, Senior Expert on Preventive Medicine (TB), Department of Disease Control, Ministry of Public Health, Government of Thailand, appreciated the efforts of WHO/SEARO in organizing the workshop. She also highlighted the need for developing country specific plans to implement effective and high quality laboratory services and surveillance for MDR and XDR-TB.

4. Technical sessions

4.1 Situation of Drug Resistance in the SEA Region

It is well recognized that effective TB control programmes, by ensuring the use of rational drug regimens have contributed to prevent and indeed, in some countries, reverse the development of anti-TB drug resistance.

The burden of anti-TB drug resistance in the Region is not well documented. A few Member countries have undertaken and reported on population-based drug-resistance surveys. Three countries in the Region, India, Nepal and Thailand, have been participating in successive rounds of the WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance. The fourth round of nationwide drug-resistance surveys (DRS) are currently on-going in Nepal and Thailand, as part of the continuing rounds of the Global DRS surveys. The country-wise status of drug resistance surveys completed since 1998 are shown in Table 1. Bangladesh conducted drug-resistance surveys in 1994 and 2001 in a rural area. The second round of national DRS in Myanmar commenced in July 2007.

Additional drug-resistance surveys are also being planned or are in the process of being conducted in Bangladesh, Indonesia Sri Lanka, and in eight additional states in India. From the available data, WHO estimated the prevalence of multidrug-resistant tuberculosis (MDR-TB) among new cases in the SEA Region to be 2.2% in 2004. Among previously treated cases, the prevalence of MDR-TB cases was estimated to be 14.9% in the same year. While a few tertiary-care facilities have reported as high as 60% levels of multi-drug resistance among previously treated cases, these are not representative of the situation in the community. The total number of cases of MDR-TB in the Region was estimated to be 114 967 in 2004, of which approximately 76% occurred in India.

Resistance to second-line drugs has been documented in the Region. The sub class of MDR-TB known as extensively drug resistant tuberculosis (XDR-TB), defined as Mycobacterium tuberculosis isolates resistant to at least isoniazid, rifampicin, any fluoroquinolone, and at least one of the three injectable drugs (amikacin, kanamycin, or capreomycin), has been isolated in samples from Bangladesh, India and Thailand. A report published by the US Centers for Disease Control and Prevention and WHO

in March 2006 revealed the presence of XDR-TB in at least 17 countries. Though not representative, the data showed that 10% of all MDR-TB isolates were XDR-TB. In the absence of any representative baseline data from Member countries in the SEA Region, an estimate of XDR-TB cannot be made. Given the widespread availability and use of second-line drugs, and as laboratory capacity to conduct second-line drugs susceptibility testing increases, additional instances of XDR-TB are likely to be identified.

Laboratory capacity for the detection of MDR and XDR-TB in the Region

The national reference laboratories at the Tuberculosis Research Centre, Chennai, India, and at the Bureau of AIDS, TB and STI at Bangkok, Thailand, are the two designated supra-national TB reference laboratories in this Region. These two laboratories are part of a global network of 25 supra-national reference laboratories. Second-line drug susceptibility testing has been performed at the Tuberculosis Research Centre, Chennai, for many years and has recently commenced at the National Reference Laboratory (SNRL) in Bangkok, Thailand.

National Reference Labs (NRLs) in all Member countries (with the exception of DPR Korea, Maldives and Timor-Leste) have capacity for mycobacterial culture. However, this capacity is quite limited even in these countries. In Nepal, culture and DST facilities are being provided through an NGO-run laboratory, quality-assured by the SNRL at Gauting, Germany. A national reference laboratory is in the process of being established. The national reference laboratories in Bangladesh, Indonesia, Myanmar and Sri Lanka are in the process of being quality assured for culture and drug susceptibility testing.

The national reference laboratories in India and Thailand are currently undertaking DST for second-line anti-TB drugs to determine the extent of XDR-TB. Reference laboratories in Bangladesh, Indonesia, Myanmar and Nepal are also engaged in rapid surveys for XDR-TB among mycobacterial isolates from patients who have failed re-treatment regimens, through linking with the SRNLs in the global network. Preliminary results from these surveys are expected to be available by end-2007 and during the first half of 2008.

4.2 Role of the laboratory, organization and management

The presentation on the TB laboratory network highlighted the primary objectives of TB laboratory services as part of national TB control programmes (NTPs), and also the need for integrating these services within the general health laboratory services. The pyramidal structure of a national laboratory with the three levels (peripheral, intermediate and central/ national) and functions at each level were elaborated. The expected size, workload and functions of laboratory staff at each level were outlined. The importance of following the recommended safety regulations was also stressed.

Laboratory safety

The session on safety in mycobacteriology laboratory dealt with equipment and facilities, policies and procedures in the mycobacteriology, personnel protection and shipment of clinical specimens and cultures. Participants were reminded of the importance of smear microscopy, as tuberculosis is almost always transmitted by smear positive patients with active pulmonary disease. Avoidance or minimizing the aerosol formation in the laboratory was stressed. Various procedures which produce infectious aerosols in the laboratory and the procedures to avoid these, such as when adding decontamination solutions, working with loops, pipetting, pouring suspensions of bacilli, and handling liquids containing TB bacilli were explained. Other hazards such as ingestion and inoculation hazards were also explained. The risk of tuberculosis infection and development of active disease by laboratory workers was highlighted. The crucial role of biological safety cabinets (BSCs), the various components and working mechanism was explained in detail. The concepts of BSL-2 and BSL-3 laboratories were introduced. After identifying some of the major constraints to the successful establishment, staffing and maintenance of BSL-3 labs, it was stressed that upgrading national labs to BSL-3 level required proper planning, financing and implementation. The presentation and discussions that followed, covered in detail the policies and procedures to be established in the mycobacteriology laboratory, the standard operating procedures for smear and culture, waste disposal, safety strategies, prevention of aerosol production and spill avoidance, spill response plan and recommended management of a spill. The importance of waste decontamination as close to the point of use as possible was highlighted. The procedures to be followed for shipment of clinical specimens and cultures were also elaborated.

4.3 Developing a comprehensive quality assurance system

The presentation was aimed at defining quality assurance (QA), internal quality control (IQC or QC), external quality assessment (EQA) and quality improvement (QI), explaining the differences between QA and QC, identification of important IQC measures for culture and DST, and listing of some important EQA norms for culture and DST. The importance of ensuring that the information generated by the laboratory is accurate, reliable and reproducible through a well defined and ongoing quality control programme, was stressed. Assessment of acceptable limits for a basic minimum quality of specimens, procedures for decontamination, digestion and culture, quality of reagents, media and equipment were discussed.

The issue of the efficacy of a culture medium and factors that contribute to the yield of positive cultures from clinical specimens was discussed. The presentation also focused on EQA for measuring the sensitivity of culture media, and recommended EQA (proficiency testing) for drug susceptibility testing (DST) for primary drugs for the purposes of diagnosis of MDR-TB and also for drug resistance surveillance (DRS).

4.4 TB Mycobacterial cultures

The main points to be considered for diagnostic tests, such as sensitivity, specificity, positive predictive value, turnaround time (speed), and implications for the individual patient that need to be considered from the programme point of view, were discussed in detail.

The advantages and disadvantages of using egg-based media for culture were explained. The yield from consecutive sputum specimens based on smear and culture examination, viability of M. tuberculosis in sputa stored at different temperatures, and effect of decontamination on the viability of mycobacteria were explained. The presentation included details of the advantages and disadvantages of the various other simple culture methods which are widely used. A meta-analysis of some of the rapid culture methods such as BACTEC, MGIT 960 and BACTEC 460 TB, with or without solid media was also presented. The flow chart for identification of M. tuberculosis was explained.

4.5 Drug susceptibility testing

The negative and positive predictive value of drug susceptibility test results was explained by comparing the frequency of probably susceptible strains against probably resistant strains inhibited at different drug concentrations. The cumulative distribution of susceptibility to isoniazid and to rifampicin through tests done in Lowenstein-Jensen (L-J) medium, using the absolute concentration method and proportion method were also reviewed. The comparison of critical concentrations of ethambutol between two different laboratories (testing done on 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. The proportion method was explained in depth as it is one of the commonly used methods for DRS. The reading of growth after four weeks of incubation (and if the growth is poor, reading again after six weeks) and reporting on the proportion of colonies grown at the critical concentration of the drug were demonstrated.

The reliability of DST results to better predict the outcomes of treatment was discussed. The importance of proficiency testing and EQA of drug media were highlighted.

4.6 Practical exercises

Demonstration and practical sessions were held after the plenary introductions and discussions on each topic.

Exercise 1: Media preparation demonstration

The participants were divided into four groups for this demonstration. Each and every step including the quality control of media preparation was demonstrated in the laboratory. The participants obtained clarifications on the finer aspects of selection of good eggs, preparation of salt solutions and L-J fluid preparation and inspissation.

Exercise 2: Sputum culture (bench work by participants)

This demonstration included safe and unsafe working practices, avoiding aerosol formation, cross-contamination and contamination of equipment.

The participants were divided into four groups for this exercise. Opportunity was provided to all participants to practice decontamination using 4% sodium hydroxide and inoculation using a simple culture method. In order to understand the effect of concentration and exposure to decontamination reagents, the participants processed two specimens, containing M fortuitum and a control. They used 1:1 and 1:2 ratios of specimen and a standardized solution of sodium hydroxide. The exposure time was 15 and 30 minutes for each. The decontaminated specimens were inoculated onto acid buffered Ogawa medium. The results of this exercise were reviewed by participants by examining the media on the fifth day of the workshop.

Exercise 3: Quality control

This exercise was intended to train participants in macroscopic identification of the quality of the media prepared.

The participants were divided into two groups for this exercise. Each participant was asked to comment, based on macroscopic examination, on the quality of the culture of media prepared for this purpose, by the host facilitators.

This was followed by inoculation of *M. fortuitum* suspensions onto two slopes of media prepared in Exercise 1, one of good quality and one of bad quality. Results of this exercise were received by the participants on the fifth day of the workshop.

Exercise 4: Calculation of resistance proportion

The objective of this exercise was to help participants learn the steps involved in calculating the different proportions of drug resistant strains within a given specimen against the four first-line, anti-TB drugs. The effects of too high or too low a density of inoculum, and the effect of contamination cultures on calculating the proportion of drug resistant strains in a given specimen, were also explained.

The participants were divided into two groups for this exercise. Each participant was made to count the number of colonies on control slopes including para-nitro beizoicacid, and on drug media at two dilutions of

bacterial suspensions, looking at media that had been prepared 28 days and 42 days in advance.

Exercise 5: Drug Susceptibility Testing

The objective of this exercise was to have the participants first observe and then perform various steps for DST such as preparation of bacterial suspensions of control (H37Rv) and INH monoresistant strains or SM monoresistant strains and test strains, setting up control (drug-free and PNB containing media) and drug containing media, inoculating suspensions on to the media, spreading the inoculum and incubating the media.

The participants were divided into six groups for this exercise.

After demonstration by SRL staff, each participant was given a set of H37Rv and INH monoresistant strain or SM monoresistant strain (2 strains for each participant).

The participants prepared bacterial suspensions and selected concentrations of test strains. They inoculated 0.1 ml each on drug-free and drug-containing media at two dilutions (10-3 mg/ml & 10-5 mg/ml) of test strains and two dilutions of control strains (H37Rv/ S resistant or H resistant), spread the inoculum and incubated the cultures. It was agreed that the host facilitator would read results after 4-6 weeks, record the results in the worksheets prepared by the participants and send these to each participant, after the workshop.

4.7 Reading and interpretation of results and reporting

The presentation dealt with the quality control aspects of pUHSDUDWLRQ RI P HGLDLn general and preparation, inspissation, sterility check, and storage of L-J media in particular. The importance of using reagent grade chemicals, following the directions carefully, not overheating the media during preparation and recording the final pH were stressed. The process for external quality control of culturing mycobacteria on egg-based media was explained in detail.

General guidelines on how to obtain good quality media were provided, covering important international quality control measures. The methods to assess quality of culture media in the laboratory network were

explained. The need for technical control at the central level, organizational responsibilities at the regional level and peripheral level were detailed. The need for annually testing commercial laboratories producing egg-based media for quality was explained. Special mention was made regarding the drug stock preparation for each of the four primary drugs — streptomycin, isoniazid, rifampicin and ethambutol. The importance of testing with known resistance strains for each drug and each new batch of medium was emphasized The presentation also described the recommended drug concentrations for streptomycin (4 mg/l), isoniazid (0.2 mg/l), rifampicin (40 mg/l) and ethambutol (2 mg/l) and setting up of the slopes for DST.

4.8 Quality control of culture and susceptibility testing for drug resistance surveillance (DRS)

The principles of anti-TB drug resistance surveillance, such as estimation, the global magnitude of drug resistance, determination of trends, evaluation of the progress of TB programmes, strengthening of laboratory networks, use of data generated for guiding policy decisions and MDR-TB management and formulation of treatment regimens were explained. The importance of ensuring sample selection in a drug-resistance survey to be representative of the population, including both new TB cases and previously treated TB cases, was explained in detail. The role of the national reference laboratories (NRLs) in ensuring quality assured laboratory results and the necessity to link with the identified Supranational Laboratory Network (SRL) was highlighted. The contents of the protocol for drug resistance surveillance (DRS) were outlined and participants invited to discuss some of the issues related to anti-TB drug resistance surveillance and testing.

Samples of M. tuberculosis should be exchanged between the SRL to the NRL for EQA or proficiency testing and from the NRL to the SRL for quality assurance of survey results for first-line drugs. Technical problems, human error, methods not being 100% equivalent are some reasons for discrepancies in results of different methods. Among the limitations is that DST is one of the most difficult procedures to perform and to standardize in the mycobacteriology laboratory. Variations in the stability of drugs subjected to different conditions of filtration, heat or storage, alteration in the activity of certain drugs when incorporated into different kinds of media, the type of susceptibility test performed, the level of experience in

reading and reporting of test results and applying the criteria of resistance all affect the reliability of drug susceptibility testing.

5. Group Discussion: Country plans for next steps

The participants worked in groups to identify issues in their own country settings, for further action to improve their culture and DST facilities, within their national laboratory networks.

The methods adopted were:

(1) Discussion by participants and facilitators for identification of issues requiring attention based on the theory and practical sessions,

(2) Review of country-specific plans developed for strengthening laboratory networks, in the Regional Consultation held in Chennai, India, in July 2006.

(3) Use of a checklist to specifically identify issues pertaining to topics covered during the workshop,

The outcomes of the group discussions are as provided in Annex 1.

6. Conclusions and Recommendations

The objectives of the workshop were met through interactive lecture-cum-demonstration sessions. Special attention was given to enhancing skills for quality assured culture and DST techniques. The fourth objective was achieved by identifying issues and actions to be taken to ensure quality diagnosis and surveillance of MDR-TB. The participants and facilitators reviewed the issues listed by them in the previous workshop at Chennai in 2006 using a specially developed instrument for identifying specific issues requiring further action in countries.

The issues identified were listed under seven broad components. Of these, some of the important issues that needed to be urgently attended were formulating country specific policies for culture and DST, ensuring assistance by SNRLs for quality assured culture and DST, for which a strong link need to be established between NRLs and SNRLs. It was also evident

during the discussions that EQA for smear microscopy was yet to be implemented in all participating countries.

It was felt that WHO should review the notified policy for culture and DST based on the estimated workload and capacity in each of the Member countries for undertaking culture and DST. There appears to be a disproportionately high number of NRLs being designated in Member countries for culture and DST. A review of the quality of culture and DST in these NRLs needed to be urgently made. Some countries (Maldives and Timor-Leste) have very low workload for culture and almost none for DST. This could therefore be done in neighbouring countries, after strengthening the mechanisms for shipping samples to neighbouring SRLs or SNRLs. Almost all countries performing culture and DST indicated their urgent need for equipment, maintenance norms, and training in quality assurance for both culture and DST.

Recommendations

- Revise the international guidelines in the light of limitations in programme capacity and lessons from the few resource-limited settings providing treatment for drug-resistant TB:
  - support flexibility in bacteriologic monitoring in view of country capacity;
  - reduce number and frequency of cultures recommended for switching from intensive to continuation phase of MDR-TB treatment;
  - reduce the frequency of smears and cultures needed for monitoring purposes, and
  - simplify and further clarify the outcome definition for cure: reduce number of negative cultures needed for classification of cure.
- WHO, Green Light Committee (GLC), technical agencies, and partners should:
  - increase availability of in-country technical assistance during preparation and early implementation phases and support resource mobilization efforts;
  - make available the Sodium-PAS formulation at the earliest;

- assist countries to build the capacity of national reference laboratories for second-line drug susceptibility testing, and
- formulate individual country targets for treatment of MDR/XDR TB in consultation with national TB control programmes.

7. Closing

On behalf of the participants, Dr Daw Ti TI of Myanmar thanked the organizers for their excellent hospitality and facilities for the workshop.

The outcomes of the workshop were summarized by Dr Pawongsak Rienttrait, Senior Medical Officer, TB Cluster, Bureau of AIDS, TB and STIs, Department of Disease Control, Bangkok. He stated that the senior TB laboratory managers were now well equipped with the needed knowledge and skills to improve and upgrade their respective laboratories, and could do so with a greater degree of confidence.

The closing remarks were made by Dr Yuthichai Kasectcharoen, Senior Medical Adviser on TB, TB Cluster, Bureau of AIDS, TB and STIs, Department of Disease Control, Bangkok. He appreciated the enthusiasm of the participants to share their skills and experience in improving the organization, management and monitoring of laboratory services. These were encouraging signs for future networking and possible collaboration among Member countries of the region.

The vote of thanks was then delivered by the Chair, Dr Tanaphan Fongsiri, Chief, ITC, Bangkok.

Annex 1

Next steps: Country Plans

	Status	Suggested next steps
Bangladesh		
Component: Laboratory network for culture and DST	National policy for culture and DST established	Awaiting MOH approval
	Plan for establishment of IRLs approved	Process ongoing
Component: Equipment – purchase and maintenance	Cold storage available	Need to install
	Incubators available	Need walk-in incubators
	Autoclaves available	Insufficient, need another one
	Other equipment required	Walk in cold room, incubator, autoclave, dessicator required
	Maintenance of equipment (Annual or Comprehensive)	Inadequate, training required for biomedical engineer
	Require help in obtaining maintenance norms	Yes
Component: Organization and practice in laboratories for culture & DST	Continuous electrical supply ensured	Requires to be procured
	BSL-3 area available in the lab	Improvement required
	Standard operating procedures (SOPs) available and displayed for all commonly performed activities	
	Training for NRL staff in culture & DST	Timeframe to be decided

	Status	**Suggested next steps**
Component: Problems frequently encountered in the laboratory – safe working practice	Spill avoidance not as per recommendation	Needs improvement
	Staff not trained in working of BSC	Staff to be trained in 2008
	Records of laboratory accidents not maintained	Staff to be trained in 2008 and records maintained
	Staff not trained in safe laboratory practices	Staff to be trained in 2008 and record maintained
Component: Quality assurance	SOP available in all sections	Being prepared
	SOP reviewed annually	Being done
	Clustering or false positives in culture evaluated	Needs improvement
	Sensitivity of culture batch checked	Preparations for this to be done- ongoing
	Sensitivity of drug media batch checked	Preparations for this to be done- ongoing
	Quarterly evaluation of contribution of culture done	Preparations for this to be done-ongoing
	Annually EQA of egg media done by NRL	Needs to be introduced in 2008
	Quality control of medium batches (H37Rv) done	Needs to be introduced in 2008
	Quality control of the validity of drug resistance criteria in the laboratory (wild strains) done	Needs to be systematically introduced in 2008
	EQA from SNRL to NRL done once a year	Being done
	EQA of IRL isolates at NRL done once a year	Needs to be introduced.
	Training requirement for NRL staff in QA	Ongoing

	Status	Suggested next steps
Bhutan		
Component: Laboratory network for culture and DST	Plan for establishment of NRL	Approved
	Plan for establishment of IRLs	Plans to be submitted
Component: Equipment – purchase and maintenance	Bio-safe centrifuge	Needs to be purchased
	Other equipment required	Additional refrigerators
	Help in obtaining maintenance norms	External technical assistance required
Component: Organization and practice in laboratories for culture & DST	BSL-3 area available in the lab	New structure to come up
	Spill response plan	Available
Component: Problems frequently encountered in the laboratory – safe working practice	Spill avoidance not as per recommendation	Practice according to guidelines
	Staff not trained in working in BSC	Training of staff
	Records of laboratory accidents not maintained	Need to be introduced
	Staff not trained in safe laboratory practices	Refresher training
Component: Quality assurance	All equipment have log	Log keeping to be introduced
	Log of equipment reviewed once a month	Needs to be systematically done
	Daily/ weekly monitoring of S+/C- done for diagnosis of patients	Need to do weekly

	Status	Suggested next steps
	Daily/weekly monitoring of contamination rates done	Being done
	Clustering or false positives in culture evaluated	In practice
	Monthly monitoring of preparation of reagents, media, handling of samples, recording and reporting evaluated	Need to introduce these procedures
	Monthly check records of pH readings of the salt solutions, sterilization records obtained, temperature monitoring devices evaluated	Need to introduce these procedures
	Annual EQA of egg media done by NRL	Needs to be introduced
	Quality control of the validity of drug resistance criteria in the laboratory (wild strains) done	To be introduced
	EQA of IRL isolates at NRL done once a year	Culture and DST planned at PHL only
	Training requirement for NRL staff in QA	DST training required
Component: Factors affecting DST results	Low level INH/ RMP resistant reference strains used for IQC	Need to review
	Training required for NRL/ IRL lab staff in DST	Training to be undertaken as planned
Indonesia		
Component: Laboratory network for culture and DST	National policy for culture and DST established	Policy development after approval by national expert committee
	Plan for establishment of NRL approved	NRL not appointed yet. Proposal will include the plan for nominating, appointing and budgeting for needed equipment

	Status	Suggested next steps
	Plan for establishment of IRLs approved	Other laboratories which are not selected as NRL will be develop as IRLs
Component: Equipment – purchase and maintenance	Bio-safe centrifuge available	Yes for three laboratories involved in ATDS survey: Additional procurements as required.
	Cold storage available	Yes for three laboratories involved in ATDS survey: Additional procurements as required.
	Incubators	Yes for three laboratories involved in ATDS survey: Additional procurements as required
	Inspissators	No, hot oven blower used in three laboratories involved in ATD survey: To be procured
	Other equipment required	Yes for three laboratories involved in ATDS survey , others not yet: additional procurements as required
	Maintenance of equipments (Annual or Comprehensive)	Partly, sources of funding limited
Component: Organization and practice in laboratories for culture & DST	Containers for safe transport specimen transportation available	Yes for three laboratories involved in ATDS survey, others not yet. Additional funding to be obtained
	Methods for rapid transport identified (courier/ postal)	Yes for 3 laboratories involved in ATDS survey , others not yet
	BSL-3 area available in the lab	Upgradation of the laboratory planned: funding to be obtained
	SOPs available and displayed for all commonly performed activities	Being finalized

	Status	**Suggested next steps**
	Spill response plan available	Partially done: need to develop guidelines and SOP
	Waste disposal as per recommendations	Partially done: need to develop guidelines and SOP
Component: Problems frequently encountered in the laboratory – safe working practice	Spill avoidance not as per recommendation	To be introduced
	Staff not trained in working in Biosafety cabinet use	To be trained at all laboratories with BSCs.
	Records of lab accidents not maintained	To be introduced
	Staff not trained in safe lab practices	To be trained at all levels.
Component: Quality assurance	All equipment have log	To finalize SOP
	Log of equipment reviewed once a month	To finalize SOP
	SOP available in all sections	To finalize SOP
	SOP reviewed annually	To finalize SOP
	Daily/ weekly monitoring of S+/C- done for diagnosis patients	To finalize SOP
	Daily/ weekly monitoring of contamination rate done	To finalize SOP
	Clustering or false positives in culture evaluated	To finalize SOP
	Sensitivity of culture batch checked	Yes for three laboratories involved in ATDS survey , others not
	Sensitivity of drug media batch checked	Yes for three laboratories involved in ATDS survey , others not yet

	Status	Suggested next steps
	Monthly monitoring of preparation of reagents, media, handling of samples, recording and reporting evaluated	Yes for three laboratories involved in ATDS survey , others not yet
	Monthly check records of pH readings of the salt solutions, sterilization records obtained, temperature monitoring devices evaluated	Yes for three laboratories involved in ATDS survey , others not yet
	Quarterly evaluation of contribution of culture done	To finalize SOP
	Annually EQA of egg media done by NRL	Will be done once the NRL is established
	Quality control of medium batches (H37Rv) done.	Yes for three laboratories s involved in ATDS survey , others not yet
	Quality control of the validity of drug resistance criteria in the laboratory (wild strains) done	Yes for three laboratories involved in ATDS survey , others not yet
	EQA of IRL isolates at NRL done once a year	Will be continued once NRL is established
	Training requirement for NRL staff in QA	Once NRL and IRLs are established
Maldives	In view of a very small number of patients requiring DST in the country, a decision regarding establishment culture facilities is to be made by the ministry	
Myanmar		
Component: Laboratory network for culture and DST	National policy for culture and DST established	Policy development after approval by national expert committee
	NRL designated	Additional funding required to support full functioning
	Plan for establishment of IRLs approved	Two laboratories identified will be developed in a phased manner

	Status	**Suggested next steps**
Component: Problems frequently encountered in the laboratory – safe working practice	Spill avoidance not as per recommendation	To prepare SOPs and provide training
	Staff not trained in working with BSCs	To give training to all staff after developing SOPs
	Records of laboratory accidents not maintained	To keep records
	Staff not trained in safe laboratory practices	To provide proper training
Component: Equipment – purchase and maintenance	Bio-safe centrifuge available	Yes, not with 3000g; to have with swing bucket
	Incubators available	Walk-in rooms to be established for Yangon and Mandalay
	Inspissators available	One each only, Need two each at Yangon and Mandalay
	Other equipment required	pH meter, densimeter to be obtained.
	Maintenance of equipments (Annual or Comprehensive)	• Need for BSCs annual check-up by Supplier • Require help in obtaining maintenance norms
Component: Organization and practice in laboratories for culture & DST	Containers for safe transport specimen transportation	To obtain containers from SNRL
	Methods for rapid transport identified (courier/ postal)	Intersectoral co-ordination between MOH and MO for transport to be established
	Continuous electrical supply to be ensured	Generator to be procured

	Status	Suggested next steps
	BSL-3 area available in the lab	Only BSL-2 in place: need to obtain BSL-3: dependent on funding
	SOPs available and displayed for all commonly performed activities	To prepare for culture & DST
	Training requirement for NRL staff in culture and DST	Need for training on culture and DST, after SOPs are developed
Component: Quality assurance	SOP available in all sections	SOPs to be developed
	SOP reviewed annually	To be introduced
	Clustering or false positives in culture evaluated	To be introduced
	Monthly check of records of pH readings of the salt solutions, sterilization records; temperature monitoring devices evaluated	To be introduced
	Quality control of the validity of drug resistance criteria in the laboratory (wild strains) done	To be done systematically
	Training requirement for NRL staff in QA	Refresher training planned in 2008.
Component: Factors affecting culture results contamination and reduced growth of MTB from sputum	Time from collection to process for culture; - Conditions of storage/ transport	Sometimes if received during late afternoon, stored in the refrigerator; storage sub-optimal. Prolonged transportation time; To inform TB Centres to send before noon.
	Harsh treatment for decontamination	NaOH concentration high and prolonged treatment time, Optimal concentration and accurate timing required
	Inappropriate centrifugation	To follow appropriate centrifugation force and time

	Status	Suggested next steps
	Expectoration of dead bacilli (specimens from patients at two/ three months of treatment)	Culture should be done only from programme point of view, not for individual treatment
	Fastidious bacilli from patient with advanced cavitary disease	To ask patients to collect sputum properly
Component: Factors affecting DST results	Potency of drugs available	Proper storage to be ensured
	Potency calculated correctly	Expiry dates to be written; reagents to be weighed precisely
	Time and temperature of inspissation correct	Electricity cut-offs during the process of inspissation - generator to be made fully functional to allow completion
	Low level INH/ RMP resistant reference strains used for IQC	To use reference stains
	Training required for NRL/ IRL lab staff DST	Refresher training in 2008
Nepal		
Component: Laboratory network for culture and DST	Link with SNRL established	Currently, linked with Genetup
	Plan for establishment of NRL approved	Planning to renovate the laboratory
	Plan for establishment of IRLs	Not indicated
Component: Equipment – purchase and maintenance	BSCs available	Three more to be obtained
	Bio-safe centrifuge available	One to be obtained
	Cold storage available	One more cold store to be established
	Other equipment required	Electronic balance, large incubators to be procured
	Maintenance of equipments (Annual or Comprehensive)	Training on maintenance

	Status	**Suggested next steps**
Component: Organization and practice in laboratories for culture & DST	Continuous electrical supply ensured	Autopower supply to be ensured.
	BSL-3 area available in the lab	Planning for BSL 3 area in the laboratory
	Training requirement for NRL staff in culture & DST	International training for 3 staff
Component: Problems frequently encountered in the laboratory – safe working practice	Spill avoidance not as per recommendation	SOPs to be developed
	Staff not trained in working with BSCs	Staff to be trained at SRNC
	Records of laboratory accidents not maintained	To be introduced
	Staff not trained in safe laboratory practices	Staff to be trained
Component: Quality assurance	SOP reviewed annually	Plans in place
	Quarterly evaluation of quality of culture done	Only at NTC: need to be introduced at other laboratories
	Annually EQA of egg media done by NRL	Only at Genetup: needs to be introduced at other laboratories
	Quality control of medium batches (H37Rv) done.	Planned renovation of laboratory
	Quality control of the validity of drug resistance criteria in the laboratory (wild strains) done	Only at Genetup: needs to be introduced at other laboratories

	Status	**Suggested next steps**
Component: Factors affecting culture results contamination and reduced growth of MTB from sputum	Time from collection to process for culture; - Conditions of storage/ transport	Immediate; cold chain, available
Component: Factors affecting DST results	Potency of drug reagents checked	To be introduced
	Potency calculated correctly	Good balance required
Sri Lanka		
Component: Laboratory network for culture and DST	National policy for culture and DST established	Includes culture and DST for all patients with positive smears at second or third month of treatment.
	Link with SNRL established	Response from SNRL awaited
Component: Equipment – purchase and maintenance	Bio-safe centrifuge available	Procurement planned
	Cold storage available	Inadequate, procurement planned
	Other equipment required	CO2 incubator, deep freeze, pH meter, Fluorescent microscope to be procured
	Each equipment has suitable voltage stabilizers	To procure back up generator and stabilizers
	Maintenance norms	Guidelines required for maintenance
Component: Organization and practice in laboratories for culture & DST	Containers for safe transport of specimens transportation available	Available
	Continuous electrical supply ensured	To be ensured
	BSL-3 area available in the lab	Planned
	Training requirement for NRL staff in culture & DST	Training of NRL staff at SNRL planned

	Status	**Suggested next steps**
Component: Quality assurance	All equipment have log	Log needs to be better maintained.
	Monthly check of records of pH readings of the salt solutions sterilization records, temperature monitoring devices evaluated	Procure pH meter and ensure monthly checking
	Annual EQA of egg media done by NRL	To be done
	EQA from SNRL to NRL done once a year	Being done
	EQA of IRL isolates at NRL done once a year	Not applicable
	Training requirement for NRL staff in QA	Planned
Component: Factors affecting culture results contamination and reduced growth of MTB from sputum	Time from collection to process for culture; - Conditions of storage/ transport	All DCCs to be provided with refrigerator
	Expectoration of dead bacilli (specimens from patients at two/ three months of treatment)	Review the policy, to change collection of specimens at second or third month of treatment
Component: Factors affecting DST results	Potency of drugs available	Is being done
	Potency calculated correctly	Is being done
	Low level INH/ RMP resistant reference strains used for IQC	Not an issue
	Training required for NRL/ IRL lab staff in DST	Training to be imparted in 2008.

	Status	Suggested next steps
Thailand		
Component: Laboratory network for culture and DST	National policy for culture and DST established	Policy development after approval by national expert committee
	NRL designated	Additional funding required to support full functioning, particularly for SNRL functions
	IRLs in place	Capacity of IRLs needs to be further strengthened
Component: Equipment – purchase and maintenance	BSC available	Available
	Inspissators available	Available
Component: Problems frequently encountered in the laboratory – safe working practice	Records of laboratory accidents not maintained	Being maintained – to be improved further
Component: Quality assurance	SOPs reviewed annually	Being done, to continue
	Quarterly evaluation of quality of culture done	Being done
	Annual EQA of egg media done by NRL	Not indicated – being done
Component: Factors affecting culture results contamination and reduced growth of MTB from sputum	Harsh treatment for decontamination	Not an issue
	Inappropriate centrifugation	Not an issue
	Training required for NRL/ IRL lab staff in DST	Staff trained
Timor-Leste	In view of a very small number of patients requiring DST in the country, a decision regarding establishment culture facilities to be made by the ministry	

Annex 2

List of participants

Country participants

Bangladesh

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Annex 3

Programme

Monday, 10 September 2007

08.30 – 09.30 Hrs **Opening**

Lecture 1 (09.30-10.00 Hrs)
Overview

- Situation of TB and drug-resistance in the SEA Region

WHO/SEARO

- Role of the laboratory, organization and management
- Implementing DRS and MDR-TB management

– Veronique Vincent

10.30 – 11.00 Hrs **Lecture 2**

Laboratory safety

- Infrastructure, equipment & maintenance
- Staffing and training
- Safe working practices
- PPE, Moving specimens & isolates

– Veronique Vincent

11.00 – 11.30 Hrs **Lecture 3**

Developing a comprehensive quality assurance system.

- What is Quality Assurance
- Internal QC
- External QA

– V. Balasangameshwara

11.30 – 12.30 Hrs **Exercise 1**

TB Mycobacterial Culture and Drug Susceptibility Testing
Media Preparation

Demonstration of media preparation

– All facilitators

13.00 – 16.00 Hrs **Exercise 2**

Sputum culture

Demonstration of safe and unsafe working practices

- avoiding aerosol formation
- cross contamination
- contamination of equipment

Participants divided into 4 groups and participate in demonstration with several participants having opportunity to practice sputum culture following the protocol

– All facilitators

Tuesday, 11 September 2007

08.30 – 12.00 Hrs **Lecture 4**

Use of culture: diagnostic efficacy, role, policy. Methods and technical issues.

- Processing sputum specimens for culture: decontamination, centrifugation, and inoculation
- Decontamination process: centrifuge, calculation of centrifugal force
- Overview of mycobacterial taxonomy
  - MTBC
  - Environmental mycobacteria and implications for TB labs
- Identification of Mycobacterium tuberculosis

– Sang Jae Kim

13.00 – 16.00 Hrs **Exercise 3**

Quality control of culture and susceptibility testing

- Internal/external quality control of culture media

– Sang Jae Kim

Wednesday, 12 September 2007

08.30 – 12.00 Hrs **Lecture 5**

Drug Susceptibility Testing¨

- Test systems, methods and critical concentrations.
- Quality issues (source of drug, storage of drug powder, storage of prepared drug concentrations)
- Safe working practice avoiding aerosol formation, cross contamination, contamination of equipment
- Preparation of drug containing media: potency calculation, dissolution, dilution, incorporation into media, and sterilization.
- Inoculum preparation and inoculation of media: bacillary dispersion, turbidity adjustment of suspension, dilution and inoculation (inoculum size standardization).

– Sang Jae Kim

13.00 – 16.00 Hrs **Lecture 6**

Reading and interpretation of results and reporting

- Quality control of drug containing media.
- Internal quality control: inclusion of reference susceptible and resistant strains
- External Quality Assessment: proficiency and cross-checking DST results.

– S. & D. Rienthong

Exercise 4

Calculation of resistance proportion

Ready to read test cultures prepared by the SRL.

- Interpretation of results of Quality Control organisms (FS, SR, HE) demonstrating different problems of DST

– S. & D. Rienthong and all facilitators

Thursday, 13 September 2007

08.30 – 12.00 Hrs **Exercise 5**

Preparation of drug containing media

– S. & D. Rienthong and all facilitators

Exercise 6

Drug Susceptibility Testing

The groups observe demonstration of setting up susceptibility tests to INH, RMP, SM, and EMB with one known INH monoresistant or SM monoresistant strain and H37Rv.

– S. & D. Rienthong and all facilitators

13.00 – 16.00 Hrs **Exercise 6 contd**

SRL to have set up 4 sets of DST for one known INH monoresistant or SM monoresistant strain (should be blinded) and H37Rv

- Participants to read the following on supplied worksheets
  - Reading at Week 4
  - Reading at Week 6
  - Reading of QC strains (FS, SR, HE)

– S. & D. Rienthong and all facilitators

Group work:

Preparation of country plans for next steps

Friday, 14 September 2007

08.30 – 12.00 Hrs **Group Discussion : Country plans for next steps**

- Laboratory network for culture and DST
- Organization and practice in laboratories
- QA

- Equipment – purchase and maintenance
- Problems frequently encountered in the laboratory – safe working practice
- Culture and DST
- Factors affecting culture results contamination and reduced growth of MTUB from sputum
- Factors affecting DST results

– S. & D. Rienthong and all facilitators

13.00 – 16.00 Hrs **Group work:**

Preparation and presentation of country plans for next steps

Annex 4

List of working papers

1. World Health Organization, SEAR, New Delhi, Situation of Drug Resistant TB in SEA Region, 2007 (draft)

2. WHO Report 2007, Global Tuberculosis Control Surveillance, Planning, Financing, World Health Organization 2007

3. STOP TB 2007-2008 XDR & MDR Tuberculosis Global Response Plan, WHO, June 2007

4. WHO, SEAR, New Delhi, Expanding Laboratory Services for TB Control, Report of a Regional Consultation, Chennai, India 11-13 July 2006.

5. Strategic Approach for the Strengthening of Laboratory Services for TB Control, 2006-2009.

6. WHO, Interim Recommendations for the surveillance of drug resistance in TB, May 2007

7. SJ Kim, Drug-susceptibility testing in tuberculosis: methods and reliability of results, Series 'Controversial issues in tuberculosis", Eur Respir J, 2005; 25: 564-569

8. Aziz M, Ryszewska K, Blank L et al, Expanding culture and drug susceptibility testing capacity in tuberculosis diagnostic services: the new challenge, Int J Tuber Lung Dis, 2007; 11 (3): 247-250

9. Espinal MA, Laserson K, Camacho M et al, Determinants of drug – resistant tuberculosis: analysis of 11 countries, Int J Tuber Lung Dis, 2001; 5 (10): 887-893

10. Kelly PM, Ardian M, Waramori G, et al, A community based TB drug susceptibility study in Mimika district, Papua Province, Indonesia, Int J Tuber Lung Dis, 2006; 10 (2): 167-171

11. T Ti, T Lewin, T T Mar et al, National anti-tuberculosis drug resistance survey, 2002, in Myanmar, Int J Tuber Lung Dis, 2006; 10 (10): 1111-1116

12. Khalezu Zaman, Zeaur Rahim, Mohammad Yunus, et al, Drug resistance of Mycobacterium tuberculosis in selected urban and rural areas in Bangladesh, Scandinavian J Inf Dis, 2005; 37: 21-26

13. Aziz MA, Wright A, Laszlo A, et al, Epidemiology of anti tuberculosis drug resistance (the Global Project on Anti-tuberculosis Drug Resistance Surveillance): an updated analysis, Lancet 2006; 368: 2142-54

14. Laboratory Assessment Report, Bhutan, 2006

15. Report of Laboratory monitoring mission to NTP, Myanmar 16-22 August 2006