

European Tuberculosis Laboratory Initiative

Regional TB and MDR-TB Diagnosis Workshop

Report

Copenhagen, Denmark, 24–25 August 2017



ABSTRACT

The European Tuberculosis Laboratory Initiative (ELI) regional TB and MDR-TB diagnostics workshop was conducted by ELI and its Secretariat at the WHO Regional Office for Europe in Copenhagen, Denmark on 24–25 August 2017. The ELI core group members meeting followed at the same venue on 25 August 2017. The workshop brought ELI core group and ELI members, including 17 heads of national tuberculosis (TB) reference laboratories of the Region, and international experts together in order to strengthen the technical capacity to diagnose multidrug-resistant TB (MDR-TB) and to advance laboratory biosafety measures using state-of-the-art techniques. The most recent developments in these fields were covered during expert presentations and were open to extensive discussions. Group work on the application of the ELI diagnostic algorithms for TB and MDR-TB and the ELI/Global Laboratory Initiative tool for interpreting and reporting the WHO-recommended line probe assay for resistance to second-line anti-tuberculosis drugs (Genotype[®] MTBDRs/VER 2) was conducted and practical experiences exchanged. The subsequent core group members meeting focused on the challenges and opportunities posed by TB/HIV co-infection and on strategies to strengthen the implementation of MTBDRs/VER 2 in the Region. The achievements of the ELI core group members during 2016–2018 were reviewed and future activities discussed.

Keywords

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Abbreviations

CDC	Centers for Disease Control and Prevention, United States
DST	drug-susceptibility testing
ECDC	European Centre for Disease Prevention and Control
ECOFF	epidemiological cut-off value
ELI	European Tuberculosis Laboratory Initiative
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FL-DST	drug-susceptibility testing for resistance to first-line anti-tuberculosis drugs
FLQ	fluoroquinolone
GLI	Global Laboratory Initiative
LPA	line probe assay
MIC	minimum inhibitory concentration
MDR-TB	multidrug-resistant tuberculosis
NGS	next-generation sequencing
PCR	polymerase chain reaction
RR-TB	rifampicin-resistant tuberculosis
SL-DST	drug-susceptibility testing for resistance to second-line anti-tuberculosis drugs
SL-LPA	line probe assay for resistance to second-line anti-tuberculosis drugs
SLID	second-line injectable drug
TB	tuberculosis
WGS	whole genome sequencing
XDR-TB	extensively drug-resistant tuberculosis

Executive summary

The WHO Regional Office for Europe and the Global Laboratory Initiative (GLI) launched the European Tuberculosis Laboratory Initiative (ELI) in 2012 to lead the scale up of the tuberculosis (TB) diagnostic capacity in response to the alarmingly high burden of multidrug-resistant tuberculosis (MDR-TB) in the Region. The WHO European Region includes nine of the 30 countries classified as having a high MDR-TB burden and 99% of the regional MDR-TB cases occur in 18 high-priority countries.

The European TB Laboratory Initiative (ELI) Regional TB and MDR-TB diagnostics workshop was conducted by the ELI and its Secretariat at the WHO Regional Office for Europe in Copenhagen, Denmark on 24–25 August 2017. The participants of the workshop came from 17 countries of the Region and included heads of national TB reference laboratories from countries of high TB priority or high MDR-TB burden, ELI regional members, ELI core group members and leading international experts.

The overall aim of the workshop was to strengthen the technical capacity in TB and MDR-TB diagnosis in the Region. The workshop provided a forum for laboratory TB experts from the Region to share experiences and to practise on ELI-developed tools. Expert presentations informed participants on biosafety and laboratory quality management systems that meet recommended standards as well as the most recent developments in the field of rapid diagnostics. Working groups debated and shared practical experiences on the use of the ELI diagnostic algorithms for the diagnosis, treatment and monitoring of patients with TB and MDR-TB, and practise sessions were held on the use of the ELI/GLI toolkit for interpreting and reporting the WHO-recommended line probe assay (LPA) for second-line anti-tuberculosis drugs (SL-LPA): the Genotype[®] MTBDR_{s/l} assay version 2 (MTBDR_{s/l} VER 2). Simultaneous translation (Russian–English) was provided for all sessions and participants from other countries were also invited to join virtually via WebEx as the meeting was web streamed live.

This report summarizes the key note presentations, panel discussions and working group experiences from this regional diagnostics workshop.

Background

TB and particularly MDR-TB remain major public health concerns in the WHO European Region. Timely and accurate laboratory diagnostic services that follow recommended biosafety measures are of key importance for controlling, detecting and treating TB and MDR-TB. Although the WHO European Region accounts for less than 5% of TB cases worldwide, about 25% of the worldwide burden of MDR-TB occurs in this Region. Of the 30 countries classified as having a high MDR-TB burden, nine are in the WHO European Region¹ and 99% of the regional MDR-TB cases occur in 18 high-priority countries.² Extensively drug-resistant TB (XDR-TB) is estimated to occur in 23.4% of all those with MDR-TB who are subjected to drug-susceptibility testing (DST) for resistance to second-line anti-tuberculosis drugs (SL-DST). Despite the fact that DST coverage has improved significantly in recent years, scaling up of testing and the use of WHO-recommended rapid molecular tests are urgently needed to reach the set goals of the regional Tuberculosis action plan for the WHO European Region 2016–2020 (TB action plan 2016–2020). Testing for rifampicin resistance among those with laboratory-confirmed pulmonary TB occurs in 44% of new cases and 49% of previously treated cases, and use of SL-DST among patients with laboratory-confirmed drug-resistant TB is 52.2%. In light of the specific needs and high MDR-TB rates in the WHO European Region, ELI with its Secretariat at the Regional Office has developed supportive tools including:

- comprehensive diagnostic algorithms for the initial TB and MDR-TB diagnosis;
- practical implementation tools for the WHO-recommended rapid molecular techniques for the detection of resistance to first- and second-line anti-TB drugs; and
- biosafety and quality assurance and managements tools for TB laboratories.

Objectives

The proposed regional workshop aimed at bringing laboratory specialists from the Region together to strengthen their technical capacity in TB and MDR-TB diagnosis and biosafety measures using state-of-the-art recommendations and techniques, with the following objectives:

- to train the participants in using the ELI core group's most recently developed TB diagnostic support tool;

¹ Azerbaijan, Belarus, Kazakhstan, Kyrgyzstan, Republic of Moldova, Russian Federation, Tajikistan, Ukraine and Uzbekistan.

² Armenia, Azerbaijan, Belarus, Bulgaria, Estonia, Georgia, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Republic of Moldova, Romania, Russian Federation, Tajikistan, Turkey, Turkmenistan, Ukraine and Uzbekistan.

- to train the participants in up-to-date knowledge and tools for TB diagnosis and TB laboratory biosafety; and
- to provide a forum for TB experts from the Region to share their expertise, experience and lessons learned.

Annex 1 lists the participants and Annex 2 gives the agenda.

Expected outputs

The expected outputs were training of the heads of national research laboratories in:

- the use of the regional ELI diagnostic algorithms;
- the use of WHO-recommended rapid molecular techniques (SL-LPA); and
- TB laboratory maintenance and biosafety.

A report describing the results of the workshop would be published on the WHO European Regional Office's website.

Wellcome and introduction

Dr Nedret Emiroglu, Director of the Division of Health Emergencies and Communicable Diseases, welcomed all participants and thanked the ELI members for their important support. She expressed her deep satisfaction with the significant achievements of the ELI group, which is setting the scene for the other regions of WHO. Although the regional TB burden is relatively small, TB remains a very high priority because of the extremely high levels of drug resistance in the WHO European Region: nine of the 30 identified countries with high MDR-TB burden are situated in the Region. On a more positive note, the Region has a strong political commitment to address the challenges posed by TB, and the TB action plan 2016–2020 has been fully endorsed and is being implemented by all Member States. ELI activities, tools and training are having a good impact on strengthening the regional laboratory network but more needs to be done. Rapid molecular testing can increase the pace of detecting cases, especially important for patients with MDR-TB, thus reducing the spread of infection in the Region. There is also much room for improvement in DST among those with confirmed TB, as the coverage is unacceptably low and well below the targeted universal access that was set in the TB action plan 2016–2020. The coverage for SL-DST among those with laboratory-confirmed drug-resistant TB in the Region is only 52.2%. The coverage for rifampicin-resistance testing among laboratory-confirmed pulmonary TB cases is 44% among new cases and 49% in previously treated cases. In conclusion, Dr Emiroglu gave special thanks to the strong contribution of the ELI core group and other members, and the support of the United States Agency for International Development was also welcomed and acknowledged.

Dr Masoud Dara, Coordinator Communicable Diseases and Programme Manager, Joint Tuberculosis, HIV/AIDS and Hepatitis Programme, congratulated the ELI core group and members on their achievements and predicted that the outcome of the meeting would have a positive impact on the Region. He reminded the group on the confluence of HIV and TB infections, which has led the WHO Regional Office for Europe to join up the programmes for TB, HIV/AIDS and hepatitis. He thanked all the participants for their efforts and for their support in the implementation of the TB action plan 2016–2020.

Professor Francis Drobniowski, Chairperson of the ELI core group and Professor of Global Health and TB at Imperial College London, emphasized the importance of the forthcoming open interactions and debate among all ELI members. He advised against becoming too complacent as drug resistance remains a very important weakness in TB programmes in the Region that must be fully and speedily addressed and resolved.

A one minute silence was observed in tribute to the memory of Dr Sabine Rüsç-Gerdes (1949–2017) as an irreplaceable TB expert within the Region, WHO consultant and former ELI and regional Green Light Committee core group member.

ELI overview of activities

Dr Soudeh Ehsani, focal point for TB and MDR-TB Laboratory Diagnostics at the WHO Regional Office for Europe, ELI Secretariat, Joint Tuberculosis, HIV/AIDS and Hepatitis Programme opened her presentation with key facts and figures on the WHO European Region, which includes 18 high-priority countries and nine high-burden countries for TB. The regional average for bacteriological confirmation of notified new cases stands at 61% and it is estimated that only 58% of all patients with MDR-TB are being detected from an estimated total of 74 000 cases and a regional target of 85%. Latest epidemiological data show that resistance to rifampicin occurs in 44% of new cases and 49% of previously treated cases and the regional coverage for SL-DST is 52.2%. XDR-TB in the Region is a cause for concern as it is estimated that 23.4% of all those with MDR-TB who are subjected to SL-DST have XDR-TB. While DST coverage has improved significantly and is better than that in some other regions of WHO, scaling up of WHO-recommended rapid molecular tests is urgently needed in order to reach the 100% target set in the TB action plan 2016–2020. The availability of GeneXpert[®] MTB/RIF (referred to as Xpert MTB/RIF) is clearly increasing among the high-priority countries (highest use in Ukraine and Uzbekistan was noted); however, the appropriate use of this test is of paramount importance in order to increase the case detection rate and to facilitate use of the short-course MDR-TB treatment regimen in the Region. Laboratory services must also keep to high-quality standards and are accountable for correct test performance and interpretation. Three priority areas of work were set for the renewed ELI core group (2016–2018) during their first face-to-face meeting in February 2016, and three products have been delivered:

- a regional diagnostic algorithm for initial diagnosis and follow up of all patients with TB;
- a technical document on a TB laboratory maintenance plan, which provides technical guidance for planning and implementing equipment maintenance in TB laboratories in the WHO European Region; and
- practical approaches and supporting tools on the correct use and reporting of WHO recommended rapid molecular tests for TB and MDR-TB.

The support tools include a training toolkit on the correct use of the WHO-recommended SL-LPA (MTBDR_s/ VER 2), which has been pilot tested in three different regional laboratories in Belarus and more recently in five regional laboratories in Kazakhstan.

Other activities have also been successfully completed, including three training sessions on the practical use of the diagnostic algorithm. These were attended by over 90 TB specialists from eight countries with high MDR-TB burden. The first and third training sessions took place at the WHO collaborating centre in Riga, Latvia, and the second in Novosibirsk, Siberia, Russian Federation. A subregional training on biosafety cabinet maintenance was conducted in Armenia in 2016, which was attended by engineers and technicians from Armenia, Belarus and the Republic of Moldova. A similar training day will be held in Tbilisi, Georgia, in September 2017 in collaboration with experts from the United States Centers for Disease Control and Prevention (CDC) and the Vladimir Regional TB Control Centre (Russian Federation).

ELI core group members and the ELI Secretariat have collaborated extensively with WHO headquarters and GLI; with other partners, including the regional Green Light Committee and European Centre for Disease Prevention and Control (ECDC); and with other regional laboratory initiatives, such as Better Labs for Better Health. In December 2016, a joint meeting between ELI members and the Better Labs for Better Health partners was held in Tbilisi, Georgia. Participants included representatives from over 20 countries in the Region and representatives from the CDC, FIND Diagnostics and the European Reference Laboratory Network for TB of the ECDC.

Part 1. ELI TB and MDR-TB diagnostic algorithms

ELI diagnostic and follow-up algorithms for TB and MDR-TB in the WHO European Region

Dr Irina Felker, Executive Director of the WHO Collaborating Centre based in the Novosibirsk TB Research Institute, Novosibirsk, Russian Federation, led the session.

This session generated a lot of exchanges between the participants and the panel, which have been summarized at the end of this section. Dr Felker distributed the printed version, in Russian and English, of the ELI diagnostic algorithm to all those present. This document represents the expert opinion of the ELI core group members and was guided by inputs from the WHO Regional Office for Europe and other former ELI members.

Dr Felker stressed that countries of TB high priority have to prioritize the use of WHO-recommended rapid molecular tests as the initial diagnostic test in both adults and children since epidemiological surveillance data show that the presence of MDR-TB can be presumed to be present. The WHO-recommended molecular diagnostic tests are LPAs and Xpert MTB/RIF. A very practical and factual summary of the preferred diagnostic methods had the following salient points.

- WHO guidelines recommend that the initial diagnosis of TB is made by rapid molecular tests (e.g. Xpert MTB/RIF) and not by conventional microscopy methods. Light-emitting diode fluorescence microscopy is the recommended method for microscopy at all levels of laboratory as this is more sensitive than the traditional Ziehl–Neelsen method.
- Culture of mycobacteria is useful for initial diagnosis of smear-negative samples, for DST, for species identification and for monitoring treatment outcome. Culture using liquid media is faster and more sensitive but solid media can also be used. Molecular amplification tests or immunochromatographic assays are recommended for species identification on culture as they provide more rapid and definite results. Automated liquid system-based phenotypic methods are the current gold standard for first-line DST (FL-DST) and SL-DST.
- Xpert MTB/RIF is based on a polymerase chain reaction (PCR) in a closed and automated system. It detects *Mycobacterium tuberculosis* and the mutations responsible for resistance to rifampicin by using three special primers and five unique molecular probes. This ensures a high degree of specificity. Xpert MTB/RIF is now the recommended method for the initial diagnosis of TB and detection of rifampicin-resistant

TB (RR-TB). The test is not useful for monitoring treatment and does not detect non-tuberculous mycobacteria.

- LPA assays such as the MTBDR_{s/l} assay detect the presence of mutations associated with drug resistance to the most important first- and second-line drugs. MTBDR_{s/l} (version 1.0) was the first commercial LPA for detection of resistance to second-line TB drugs. Version 2.0 was marketed in 2015 and has been recommended by WHO since 2016 to identify resistance to fluoroquinolone (FLQ) and second-line injectable drugs (SLIDs).
- FL-LPA can be used on sputum-positive and culture-positive samples. Results are highly sensitive ($\geq 97\%$) and specific ($\geq 99\%$) for the detection of RR-TB, alone or in combination with isoniazid resistance (sensitivity $\geq 90\%$; specificity $\geq 99\%$).
- SL-LPA can detect 86% of patients with FLQ resistance, 87% of patients with SLID resistance and 69% of XDR-TB when used on positive sputum samples (direct testing). It has a lower yield on smear-negative samples and does not distinguish between resistance to individual FLQs (but has a high correlation with phenotypic resistance to ofloxacin and levofloxacin).
- There are several advantages to genotypic molecular methods, including reliable diagnosis within a few hours compared with up to six weeks for standard culture results, standardized methods producing highly reproducible results, potential for high throughput, lower biosafety hazards for staff, and highly valid and reliable results.

The ELI diagnostic algorithm focuses on three components: (i) the initial diagnostic work flow of all suspected cases of TB, (ii) the follow up of patients with drug-sensitive TB, and (iii) the follow-up of patients who are found to have MDR-TB. Dr Felker made some practical comments that would be useful for national and regional reference laboratory staff on the interpretation and reporting of laboratory results. She stressed that genotypic results should be reported directly to the attending clinician so that the correct treatment is given without delay. As discordant results between phenotypic DST and genotypic DST can and do occur in practice, laboratory errors must first be excluded. Staff should be well versed in molecular biology sciences so that they can confidently explain these discrepancies to clinicians. Possible explanations include silent mutations, rare or disputed mutations, mutations out of the hotspot region for the test and heteroresistance (mixed infections can occur in countries with a high MDR-TB rates). Whole genome sequencing (WGS) could provide the final answer in such situations; however, this is still not widely used for clinical purposes in TB diagnostics and is not available in many countries.

The topics presented led to a lively debate and the main points raised included the following general topics.

Work load implications. The capacity of laboratories to accommodate the implementation of new tests such as SL-LPA was raised by several participants. They were concerned that human resources are already stretched and cannot accommodate the extra workload. A large volume of samples is already received by laboratories as clinicians request repeated testing of multiple samples to monitor treatment outcome.

Communication. Better communication between laboratory staff and clinicians is called for. Each national TB programme has to decide its own protocol for the periodicity of repeat testing to monitor treatment, but repeat DST is not necessary on every sample. This should only be requested if there is a strong clinical suspicion, such as persistence or reversion to sputum-positive result. Clinicians should be informed that it is useless to request a repeat Xpert MTB/RIF if a previous test has already detected RR-TB. An SL-LPA test is warranted in this situation. Clinicians should also be aware that SL-LPA detects resistance mutations only for groups A and B anti-TB drugs (i.e. FLQ and SLID). Phenotypic DST is still necessary for groups C and D anti-TB drugs.

Assure quality. While many laboratories in the Region use two samples for each round of testing, laboratories with good quality standards are using only one sample. Consequently, assuring high-quality standards can have direct implications on the workload. Some laboratories in the Region freeze the original samples and these are retested if an indeterminate result is obtained, thus avoiding the need to obtain new samples.

Sample transportation system. Where GeneXpert[®] machines should be placed in countries of high TB priority was raised following the scale up of decentralized people-centred care. Patients may have to travel long distances if the test is only performed at central TB laboratories; consequently a sample referral system should also be part of health system restructuring.

DST for new drugs and generic PCR tests. The question of generic PCR-based diagnostic techniques was raised since a few are emerging in the market. Some reasons were given to refute the use of a generic method, such as doubtful quality control, non-disclosure of the primary probes used and insufficient published scientific investigation of performance standards. Endorsement by WHO is a strong signal that a technique has been very arduously assessed against strict standards in an open and transparent manner by a team of experts in the field.

Group work session on case studies using the ELI algorithms

Dr Irina Felker was the instructor for the group work.

Dr Gulmira Kalmambetova, Head of the National Reference Laboratory, Kyrgyzstan and ELI core group member, and **Professor Sven Hoffner**, Karolinska Institutet, Sweden and ELI core group member, were group work facilitators.

A set of seven clinical scenarios were distributed among the seven working groups together with a copy of the diagnostic algorithm. The working groups were asked to apply and discuss the ELI algorithm with their assigned case. The country perspectives for Cases 1, 4 and 6 were then very briefly presented and a general discussion by all the participants ensued. All case studies were available in Russian and in English and are included in Annex 3 of this report.

Group work presentations and discussion

Case 1: perspectives from Latvia and Croatia

The group assigned to this case presented two very contrasting positions: a high-priority country (Latvia) and a low-burden country (Croatia).

Latvia. Two samples are received for each round of testing and the initial laboratory form usually includes a request for both direct microscopic examination and Xpert MTB/RIF testing. Positive sputum samples are tested by Xpert MTB/RIF on the same day and the clinician is informed of the result on the same day. If RR-TB is detected, then FL-LPA for isoniazid sensitivity and SL-LPA are automatically performed. An expert consilium decides the appropriate treatment regimen and also reviews cases with discordant results. Sputum samples from HIV-positive patients are tested by Xpert MTB/RIF irrespective of the direct smear result.

Croatia. Xpert MTB/RIF is not performed for the majority of patients because only 3% of samples tested in the country are positive for TB. The low HIV prevalence rate in the general population is another important determinant. Only one case of rifampicin resistance has been detected since the test became available in the country. This low positivity rate has important cost implications. Laboratories are reimbursed only if the physician sends a request for the test.

Case 4: perspective from Kyrgyzstan

Regional laboratories process samples from patients with TB, and two samples are received for each round. Xpert MTB/RIF is used for diagnostic purposes. Direct microscopy is performed on all samples and Xpert MTB/RIF is performed only on one sample. Invalid results do occur and this test is repeated from the same sample whenever possible, although samples are not routinely

stored. If RR-TB is detected, SL-LPA is performed. FL-LPA is also available to detect resistance to isoniazid. The final treatment regimen takes into account the results from both genotypic and phenotypic DST methods.

Case 6: perspective from Lithuania

Laboratories generally receive three samples for each testing round. Direct microscopy testing is performed in all three, and two samples are used for solid cultures. Staff members keep a close watch on the progress of patient test results and alert clinicians immediately if a culture reverts back to positive. They also monitor closely if microscopy is still positive by the end of the second month of treatment. FL-LPA is done if samples are still positive by the third month and SL-LPA is suggested if necessary.

Summary of exchanges and discussion from all participants

- The cost of performing Xpert MTB/RIF was discussed, and it was concluded that the cost to countries of misdiagnosis or late diagnosis of MDR-TB is clearly higher. Some countries are now increasingly focusing on molecular methods because phenotypic DST for first- and second-line drugs may be more expensive.
- Laboratory staff may face a dilemma if they see the need to use a rapid diagnostic method without having a specific request from the attending physician. Most participants said that they would confidently discuss individual test results with clinicians if warranted and that the interest of patients should be given the highest priority. They also agreed that many clinicians are still not fully aware of the high validity of molecular test results and increasing awareness among physicians was suggested as laboratory staff may not have the authority to direct or influence clinical decisions on the chosen diagnostic test.
- A case in point is the need for pooling samples and conducting batch testing for LPAs, resulting in clinicians receiving results from SL-LPA and phenotypic DST often at the same time.

Keynote session: discrepancies between genotype and phenotype

The session focused on what antimicrobial resistance is and how it is defined (critical concentrations and minimum inhibitory concentration (MIC)³).

³ The minimum concentration of drug that causes inhibition of 99% of pathogen growth, with a variation of ± 1 dilution.

Dr Claudio Köser, Wolfson College Research Associate, University of Cambridge, United Kingdom led the session.

Declaration of interest: Dr Köser has worked with the Foundation for Innovative New Diagnostics (FIND), the Bill & Melinda Gates Foundation and PerkinElmer. He has collaborated with Illumina Inc. and previously received travel funding from Janssen Pharmaceutica. He received the Gertrud Meissner Award from the European Society of Mycobacteriology, which was sponsored by Hain Lifescience.

Dr Köser opened his session by reminding participants that although mycobacterial resistance to anti-TB drugs is traditionally defined by phenotypic DST, this method is based on a number of assumptions and a limited understanding of resistance mechanisms. Consequently, discrepancies between genotypic and phenotypic DST results for anti-TB drugs can occur and often cause confusion. He stressed that clinicians sometimes choose to disregard the significance of discordant results, assuming poor quality of the laboratory service, and also mentioned that unclear results appear to be more common in certain regions. He explained that phenotypic DST can have poor reproducibility for resistant isolates because the phenotypic wild-type and non-wild-type distributions for TB are uniquely close or even overlapping for several antibiotics. In these situations, genotypic DST can be a better way to detect resistance caused by known resistance mechanisms, and disregarding genotypic DST results may be even harmful to patients. Dr Köser gave a brief overview of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) approaches on the definitions of MIC, the epidemiological cut-off value (ECOFF),⁴ the clinical breakpoint⁵ and critical concentration (WHO approach is that this is equal to ECOFF). Dr Köser stressed that some variation is inherent in MIC testing, which is taken into account in the EUCAST approach to systematically define breakpoints for most clinically relevant bacterial pathogens. By contrast, the critical concentrations that define resistance in TB were set up largely based on expert opinion in the past, which means that breakpoint artefacts (i.e. inappropriately high critical concentrations) have resulted in the systematic misclassification of resistant strains as being susceptible for some drugs. This source of error can be addressed by lowering the critical concentration to the breakpoint value. However, the reproducibility of testing for resistance mutations that only confer slight MIC increases (i.e. where the MIC distribution of susceptible isolates overlaps significantly with the distribution of resistant strains) remains poor even if the critical concentration is equal to the ECOFF. Kanamycin resistance

⁴ ECOFF, or microbiological breakpoint, corresponds to the highest concentration of the phenotypically wild-type MIC distribution and encompasses 99% of phenotypic wild-type isolates. Strains with MIC > ECOFF are referred to as phenotypically non-wild-type.

⁵ MIC value that distinguishes organisms of the same species, or closely related species, that are likely to succeed therapy from those that are likely to fail, i.e. sensitive/intermediate/resistant.

caused by *eis* mutations, which are frequent found in countries of the former Soviet Union, are a good example of this phenomenon.

Special mention was also made of the assumed reproducibility of MIC and that the correlation with treatment outcome is still not clear for all anti-TB drugs. Dr Köser also referred to lack of a database for mycobacterium TB doses and noted that anti-TB drugs are often too toxic to be used at the higher doses needed to reach clinical breakpoint values. In addition, reference MIC levels can differ between different agencies (e.g. WHO, the United States Food and Drug Administration). He also explained that, in practice, several factors can lead to discordant results, including laboratory error in either the genotypic or phenotypic test method used; a breakpoint artefact if the MIC value is greater than the ECOFF value; or "silent" mutations, when the mutation causes only a small increase in MIC.

While expert guidance is being developed to address such situations, Dr Köser suggested that:

- if a resistance mutation is specifically identified using a valid genotypic assay, the isolate should be reported as resistant;
- phenotypic DST should *not* be performed as there is a significant probability that the isolate would test phenotypically susceptible because of the overlapping MIC distributions;
- if phenotypic DST is performed for whatever reason, a genotypic-resistant result should overrule a phenotypic-susceptible result (provided that experimental error is excluded); and
- phenotypic DST is still needed for isolates that lack resistance mutations.

Key note session: role of genome sequencing for TB and MDR-TB diagnosis

Dr Philip Supply, CNRS, Centre for Infection and Immunity of Lille, France, discussed the role of genome sequencing.

Declaration of interest: Dr Supply is a member of the Scientific Advisory Board at GenoScreen. Dr Supply stated that serious detection and treatment gaps still persist in many countries as it is estimated that on a global scale <30% of MDR-TB cases are being diagnosed and <50% of these cases are being treated. This problem is compounded by the fact that standard DST for mycobacterial cultures is very slow and the more recent rapid molecular tests are limited as they interrogate only a limited fraction of mutations that are commonly associated with drug resistance. WGS holds great promise for predicting drug resistance as it provides nearly

comprehensive access to the genetic information of the pathogen and correctly identifies mycobacterial species. In addition to its value as a diagnostic tool, WGS is useful for epidemiological surveillance, in outbreak investigations and also as a basic research tool (e.g. by assessing the virulence of a specific strain). WGS, however, is also limited as it still relies on obtaining a primary culture from clinical isolates to predict drug resistance and is not used directly on clinical samples. Consequently, it is slow, requires costly selective enrichment methods and yields low genomic coverage. The techniques used are complex and not standardized, and reporting of results is not user friendly. A recent systematic review of WGS concluded that although this is promising for detecting resistance to the main first-line drugs and to FLQs, there is much need for a comprehensive resistance mutation/gene database for other drugs.⁶ The latter is being addressed by project CRyPTIC, which is attempting to build a large genome database to lead the prediction of susceptibility and resistance patterns on a global scale. In Dr Supply's opinion, targeted next-generation sequencing (NGS) can bypass some of these limitations. A number of platforms are available on the market with varying levels of portability and affordability. Second-generation assays rely on amplified fragments and the third generation can read up to single molecules. Some examples of second-generation modules that are useful in routine clinical applications were mentioned (e.g. Thermo Fisher/Vela Ion PGM/55™, Illumina MiSeq-Miniseq™ and Qiagen GeneReader™). NGS uses DNA that is directly extracted from clinical samples, requires lower genomic inputs by using PCR amplification, can detect heteroresistant populations and can test up to 90 samples in one testing run. It has great potential in epidemiological surveillance of drug resistance and for initial triage of suspected XDR-TB in countries with limited laboratory capacity. It is, however, very important to note that NGS can still miss minor or unknown targets and genotyping information remains very limited. Dr Supply described in detail the Deeplex®-MycTB all-in-one NGS-based diagnostic assay for mycobacterium TB, which has been field tested in Djibouti. The assay uses ultradeep sequencing of a single 24-plexed amplicon mix for simultaneous mycobacterial species identification, genotyping and prediction of drug resistance of *M. tuberculosis* complex strains. More than 40 samples can be analysed in one MiSeq™ run. The test simultaneously analyses 18 main gene targets associated with first- and second-line drug resistance. The achieved high coverage depths enable detection of low-frequency variants that can cause clinical resistance, and results are derived using integrated databases that compile reference results from large WGS-based studies. Deeplex®-MycTB makes use of cloud-based visualization tools that allow for quick and user-

⁶ Papaventsis D, Casali N, Kontsevaya I, Drobniowski F, Cirillo DM, Nikolayevskyy V (2017). Whole genome sequencing of Mycobacterium tuberculosis for detection of drug resistance: a systematic review. Clin Microbiol Infect. 23(2):61–8.

friendly interpretation and reporting of the NGS results (e.g. by including colour-coded visualization). The results were also highly reproducible with different sequencing chemistries and different NGS platforms, such as Qiagen GeneReader™ and Illumina MiSeq™.

The discussion that ensued appeared to be concentrated around the cost of the next-generation WGS tests and on the paucity of TB genetic data from across the Region.

Cost. The cost of WGS needs to be considered but, in general, the cost of these new tests is getting cheaper and may be well within the reach of many countries. However, the calculated capital and recurrent costs in some countries that are already using this technique may be artificially distorted (e.g. special budgets are allocated to reference and research laboratories in the United Kingdom). Many countries of the European Union/European Economic Area still do not have access to routine WGS testing, but there appears to be rapid progress in this direction. Projects with international partners (e.g. the KNCV Tuberculosis Foundation of the Netherlands or the United States National Institutes of Health) may be useful to gain some experience on the use of WGS in high-priority countries, but this may have limited sustainability. Experts are debating the use and added value of sequencing technologies for clinical purposes during similar meetings of countries of the European Union/European Economic Area (ECDC and European Reference Laboratory Network Tuberculosis).

Collating genetic data. A genetic data repository of mycobacterial strains is a useful resource. Countries that are high priority for TB have much to offer as they have numerous cases with diverse resistance patterns, but they do not have the resources to enjoy the benefits of WGS. WHO has so far not included WGS in any of its recent updated guidelines.

Part 2. TB laboratory maintenance and biosafety

ELI TB laboratory maintenance plan

Dr Natalia Shubladze, Research Consultant, National Centre for Tuberculosis and Lung Diseases, Tbilisi, Georgia and ELI core group member, gave a presentation on the ELI-developed TB laboratory maintenance plan (LMP). The document represents the expert opinion of ELI core group members and provides practical guidance on the maintenance of equipment in TB laboratories in the WHO European Region and stresses efficiency and biosafety. This document is available in Russian and English and reaches a diverse target audience, including laboratory managers, managers of national TB programmes, donors and other partners. It describes the elements that are needed for the smooth functioning of laboratories, assigns responsibility and provides a stepwise approach to implementing an equipment maintenance plan. Specific details are included for a full range of TB-related key laboratory equipment. It encourages the use of a log book for recording and updating the laboratory inventory and it also describes standard operating procedures. The concept of a "book of life" is introduced, where full details are recorded for the lifespan of the hardware and all the repairs are recorded. A practical maintenance plan template is also included in the document.

The brief discussion clarified the following two general areas of interest.

Maintenance versus repairs. Regular maintenance is normally performed by laboratory staff while repairs require external engineers/technicians to solve a problem. Engineers need to meet specific requirements and warranties may be affected if repairs are not performed by a qualified technician who is recognized by the manufacturer. Many countries have bidding procedures to obtain external repairs and these often can take a very long time to be completed.

Coordinated planning. The LMP document is also intended to inform managers of national TB programmes and should assist in forward budgeting and planning. The capacity of qualified engineers is increasing as a result of specific training courses led by the WHO Regional Office for Europe.

TB laboratory biosafety

Dr Grigory Volchenkov, Vladimir Centre of Excellence for TB Infection Control, Vladimir, Russian Federation, opened his presentation by reminding participants that biosafety is the discipline on the safe handling and containment of infectious microorganisms and hazardous biological materials in laboratories and stressed that biosafety standards are critical for all laboratories. WHO has published a TB Laboratory Biosafety Manual in order to guide low-

income countries to implement high biosafety standards and this contains practical information on how to conduct a procedural risk assessment for TB laboratories. It also defines the minimum requirements necessary to mitigate risks (e.g. prevent exposure of staff to TB from aerosols), grades risk into low, medium or high (e.g. manipulation of TB cultures and DST is high risk), and gives advice on how to mitigate risk with the correct use of biological safety cabinets.

Dr Volchenkov covered a range of biosafety equipment that is used in TB laboratories and other related health care settings, and a summary of the points on the different equipment types is included below. Full details are available in the WHO TB Laboratory Biosafety Manual, which is included in the Suggested reading at the end of this report.

Biological safety cabinets. This equipment has strict ventilation requirements and canopy/thimble fittings that should be specifically included in the terms of reference during procurement. The ventilation conditions for high-risk TB laboratories must include the following standards: 6–12 air changes per hour; unidirectional airflow; negative pressure of at least 2.5 Pa; air velocity in occupied areas <0.5 m/s; air conditioning (air recirculation is not recommended); thimble connection to exhaust duct (5 cm gap, +20% flow). Biological safety cabinets must adhere to set certification standards (EN12469-2000 or NSF/ANSI49-2009) and should be professionally installed and maintained. Dr Volchenkov gave some practical examples of simple measures that are useful for routine monitoring. He cautioned that molecular diagnostic methods are sensitive to high room temperatures and that poorly drained air conditioning units created a risk of legionella infection. A number of factors affect the performance of these safety cabinets, and the audience was shown a set of photographs from different laboratories to reveal poorly installed equipment. Some common shortcomings included the cabinets being placed in a high traffic area or very close to doors or open windows. Dr Volchenkov stressed that negative pressure outlets should not be adjacent to the air-conditioning compressor; autoclaves should not be placed in low-risk areas, and airflows should be in the correct direction. A smoke test is an easy and convenient method to assure that air is flowing in the right direction.

Room air cleaners. Dr Volchenkov said that he has commonly encountered these devices in high-risk settings in many parts of the Region and that several of them are manufactured locally. Units are electrically operated, free standing or portable and work by removing or deactivating airborne contaminants from the air. He observed that manufacturers stress the ease of use and guarantee the safety provided by them, but in his opinion their use can be problematic as advertised standards are not met in reality. A number of such poor

examples from different countries were presented, and some models were described as nothing more than a "UV lamp in a box". He reminded participants that manufacturers must fully disclose important specifications such as the airflow rate, the capacity (how long the unit can operate before cleaning or replacement is necessary), the particle collection efficiency (a function of particle size) and the clean air delivery rate. Units can have fibrous filters (including HEPA filters), ultraviolet germicidal irradiation, electrostatic precipitation, negative ion generators, photocatalytic oxidations and some other hybrids. Other important factors that need to be considered before acquiring such units are the clean air delivery rate, the equivalent air changes per hour, capital and maintenance costs, sustainability, safety and comfort.

Personal respiratory protection. Dr Volchenkov considered that respirators are not normally required for work in a TB laboratory but may be recommended after a risk assessment if TB cultures are being manipulated. Respirators can be either disposable or reusable, are available in different sizes and should conform to set standards that describe the proportions for filter penetration and total inward leakage (United States Standard NIOSH N95 or FFP2 European Standard EN149:2001). Dr Volchenkov noted that FFP2 or FFP3 respirators are recommended for settings of high TB transmission risk (or N95 of United States standard 42CFR84). He also stressed that respirators must be fit tested and staff in high-risk situations should have annual fit testing; users can easily check the seal by seeing the respirator collapse on inspiration. In addition, disposable respirators should be discarded if damaged or wet. Elastomeric face piece respirators can be reused, after cleaning and disinfection, and he cautioned that filters should be replaced if damaged, contaminated or causing excessive resistance while breathing. Surgical masks are not certified and should not be used as respirators.

Part 3. MDR-TB diagnosis using WHO-recommended MTBDR_s/VER 2

MTBDR_s/VER 2 line probe assay to detect resistance to second-line anti-TB drugs

During this joint session Dr Soudeh Ehsani briefly reviewed the training toolkit that was developed by the ELI in collaboration with GLI and Dr Shubladze gave more details on result analysis, interpretation and reporting of test strips.

MTBDR_s/VER 2 is based on DNA STRIP[®] technology or DNA LPA and can be used to diagnose MDR-TB and XDR-TB. It detects mycobacterium TB and the presence of mutations that are associated with resistance to FLQs, aminoglycosides and cyclic peptides in SLIDs. MTBDR_s/VER 2 does not detect resistance to ethambutol but can be performed directly on sputum (negative or positive) samples and on cultures of mycobacterium TB. The ideal turnaround time for this test is five working days.

Policy recommendations

WHO has issued policy recommendations on the use of MTBDR_s/VER 2,⁷ which are summarized as follows:

- resistance-conferring mutations detected by SL-LPA are highly correlated with phenotypic resistance to ofloxacin and levofloxacin but the correlation of these mutations with phenotypic resistance to moxifloxacin and gatifloxacin is unclear and the inclusion of these two drugs in an MDR-TB regimen is best guided by phenotypic DST results;
- resistance-conferring mutations detected by SL-LPA are highly correlated with phenotypic resistance to SLIDs and are an indication to use an appropriately strengthened MDR-TB regimen; and
- given the high specificity for detecting resistance to FLQ and SLIDs, the positive results of SL-LPA could be used to guide the implementation of appropriate infection control precautions.

⁷ The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: policy guidance. Geneva: World Health Organization; 2016 (<http://www.who.int/tb/WHOPolicyStatementSLLPA.pdf>, accessed 22 October 2017).

Test accuracy

By direct testing, SL-LPA will detect:

- 86% of patients with FLQ resistance and will rarely give a positive result for those without resistance;
- 87% of patients with SLID resistance and rarely gives a positive result for patients without resistance; and
- 69% of patients with XDR-TB and rarely gives a positive result for patients without resistance.

Strip description

Each strip has a total of 27 reaction zones covering six areas of interest and test protocols and procedures must be strictly followed in order to obtain correct test results and to avoid contamination. Dr Shubladze listed a number of important observations for each and the following are some salient points:

- conjugate control: a line must develop in this zone as it is an indicator of the efficiency of conjugate binding and substrate reaction.
- amplification control band has to be positive even in negative control specimens. It indicates that the amplification reaction was correct and not inhibited:
 - the test should be repeated if the band is missing; and
 - all bands (except the conjugate control band) should be compared with the amplification control band for density, with bands that are less dense not reported.
- *M. tuberculosis complex* control: if this band is negative and no evaluable resistance pattern is developed, the tested bacterium does not belong to the *M. tuberculosis* complex and cannot be evaluated by this test system.
- Locus controls detect a gene region specific for the respective locus (*gyrA*, *gyrB*, *rrs* and *eis*):
 - if the locus control zones are negative, then their respective mutation-specific positive bands cannot be considered for evaluation; and
 - if all bands including the locus control band are missing, this indicates an invalid result (e.g. DNA concentration in the sample below the limit of detection).

- Wild-type probes examine *gyrA*, *gyrB*, *rrs* and *eis*:
 - they cover the most important resistance regions of *gyrA*;
 - if all wild-type probes stain positive, and there is no detectable mutation within the examined region, then the strain is sensitive for the respective antibiotic (*gyrA* and *gyrB* for FLQ, *rrs* and *eis* for SLIDs); and
 - absence of wild-type bands imply that the strain is resistant, even if mutation bands are absent.
- Mutation probes detect some of the most common resistance-mediating mutations (*gyrA*, *gyrB*, *rrs*, *eis*).

Interpretation of results

- Negative control serves for detection of contamination and should be set for every batch; a negative control strip should have only two bands, conjugate control and amplification control.
- Wild-type column is reported as:
 - "+" if all wild-type bands of a locus display a signal of the same intensity or as strong as the amplification control; and
 - "-" if at least one of the wild-type bands is absent.
- Mutation column is reported as:
 - "+" if at least one of the bands is manifested stronger than amplification control; and
 - "-" if the band is weaker than the amplification control.

Other points to note

- Most mutations leading to FLQ resistance have been identified in a conserved region called the quinolone-resistance-determining region of *gyrA* (320 bp) and *gyrB* (375 bp);
- Both *gyrA* and *gyrB* are examined for detection of resistance to FLQs (i.e. ofloxacin, levofloxacin, moxifloxacin and gatifloxacin);
- *rrs* is examined for detection of cross-resistance to aminoglycosides/cyclic peptides antibiotics such as kanamycin, and amikacin, and capreomycin; and

- Mutations in the *eis* promoter region confer low-level resistance to kanamycin; in the presence of a concomitant mutation in *rrs*, the strain is highly resistant to this drug, regardless the presence of mutations in the *eis* promoter region.

Test limitations

The test has a number of limitations that must be borne in mind.

- It only screens the nucleic acid sequence and not the amino acid sequence. Consequently, it is possible that mutations in the probe region that do not cause an amino acid exchange (silent mutations) will still produce the absence of one of the wild-type bands.
- It only detects resistances that have their origins in the *gyrA*, *gyrB*, *rrs* and *eis* gene regions and so resistance originating from mutations of other genes or gene regions will not be detected.
- Results must be interpreted in combination with additional laboratory and clinical data and the results of phenotypic DST have to be considered in certain cases.
- MTBDR_s/ VER 2 is a qualitative test and the intensities of the bands on a strip do not give information about the number of cells in a positive sample; therefore, it has no correlation with infectivity. It may not be used for monitoring response to treatment.
- Members of the mycobacterium TB complex cannot be differentiated and the presence of multiple bacterial species in the sample might hamper interpretation of the test.
- The user must have or acquire information about the local mutation distribution pattern of the genes investigated with this test. Confirmation of the test results by phenotypic DST may be necessary.

SL-LPA training toolkit

Major elements of the SL-LPA training toolkit on the correct use of the WHO-recommended MTBDR_s/ VER 2 were reviewed during this session. This technical document was developed as a follow-up to the ELI diagnostic algorithms, which stress the importance of the rapid detection of MDR-TB and increasing access to DST using SL-LPAs. It covers performing the test, analysing and interpreting results, recording and reporting results to clinicians using a purposely designed template and targeted troubleshooting. It suggests a check list of laboratory requirements. The tool was first pilot tested in Belarus in three different regional laboratories and real examples of test results were used in the training for exercise purposes. This pilot training

was very useful as some knowledge gaps were identified that have since been addressed in the final document.

Group work session on test analysis of examples from the field

After the detailed description of the test and test result analysis, participants were divided into working groups on interpretation and troubleshooting and given practical examples of test results to discuss and analyse, followed by presenting their conclusions to the floor. The following represents the main points that were raised by the groups.

Results described as showing "low-level resistance". Some participants expressed the view that using this terminology in laboratory reports may be misleading. Clinicians may choose to increase the dose of the identified drug, which is not correct and not recommended. Such cases are best discussed between the laboratory experts and attending clinicians. The need for more guidance given by WHO was expressed.

Mapping common mutations. The group discussed and agreed that it would be very useful if the results that are being generated could be recorded and tracked. This would be a unique opportunity to observe the frequency, distribution and spread of specific mutations that are occurring in the Region. It would also be useful to validate and interpret results in some instances.

Views from clinicians. The results determine the final outcome of treatment and, therefore, it is important that they are accepted by the attending clinicians. Results must be clearly worded and include a clear conclusion as the expert view from the laboratory. The toolkit helps to simplify the interpretation and reporting of the test, and more practical versions can be drafted in the future that are tailored for clinicians. It was also suggested that more information is added on the result form to include the FL-DST results.

Test performance. Practical experiences from some participants revealed that the results from negative smear sputum samples and from scanty samples (even if smear positive) were disappointing.

Difficult interpretations. In practice some test strips can be difficult to interpret, for example reagents may fail to bind or bands can be too faint. In such situations, the test would have to be repeated.

Closing remarks

Dr Masoud Dara and Professor Francis Drobniewski closed the meeting by thanking all those present for their very active participation and insightful contributions. Efforts must continue to broaden the dialogue between laboratory services, clinicians, researchers and other partners in the Region. Finally, participants were reminded of the upcoming first WHO Global Ministerial Conference that will be held in Moscow, Russia in November 2017, which is an important event that will accelerate the implementation of the END TB strategy of WHO.

Conclusions

At the end of the two days, participants were asked to evaluate the workshop using a structured questionnaire that was available in both English and Russian. A total of 25 responses were collected from among the 27 participants. The workshop content was rated very highly overall for content and design, but one respondent strongly disagreed that the difficulty level was appropriate or that the objectives were clearly communicated (Annex 4). The facilitators were considered to be well prepared; however, the participants would have preferred more time dedicated to discussions of the case studies. The majority also strongly agreed that the workshop was a good venue to learn, that the material presented was useful and that they would apply the knowledge acquired at their place of work.

The following main conclusions were drawn from the workshop meeting.

- Laboratory specialists must take a more active role in clinical patient management and work even more closely with clinicians. They have the expertise on the performance and limitations of the new testing methods. Legal constraints in some countries may, in fact, limit such a direct contribution but can be overcome by open communication in most instances.
- The rapid technological advances that are occurring in the field of molecular diagnostic testing, genotypic DST and WGS can outpace the technical comprehension of both laboratory staff and clinicians. It is important that clinicians be included in both regional and country training sessions on the practical use and interpretation of these new techniques.
- Basic science research is increasing the understanding of resistance-causing mutations.
- The interpretation of discordant results or low-level resistance can be confusing. Laboratory specialists need additional training, and communication between laboratory experts and attending clinicians can lead to more informed clinical decision-making.
- Investment in new technologies needs to consider the epidemiological situation in the country as well as potential cost savings from other sectors in the health service (e.g. reduced hospital admission costs, reduced consumption of ineffective drugs and reduced transmission of MDR-TB in the community).
- Targeted WGS is a recent advance and holds good promise but the confidence and validity of results still depend on testing highly positive sputum samples and thus indirectly is based on the sensitivity of microscopy.

Suggested reading

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Annex 2. Meeting agenda

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REGIONAL OFFICE FOR EUROPE



ORGANISATION MONDIALE DE LA SANTÉ
BUREAU RÉGIONAL DE L'EUROPE

WELTGESUNDHEITSORGANISATION
REGIONALBÜRO FÜR EUROPA

ВСЕМИРНАЯ ОРГАНИЗАЦИЯ ЗДРАВООХРАНЕНИЯ
ЕВРОПЕЙСКОЕ РЕГИОНАЛЬНОЕ БЮРО

European TB Laboratory Initiative (ELI) Regional TB and MDR-TB diagnostics workshop	1/2
Copenhagen, Denmark	Meeting room: Auditorium 1
24–25 August 2017	Original: English

Programme 24 August

Time	Topic	Speaker
08:30–09:00	Registration	
09:00–09:30	Welcome and Introduction	Dr Nedret Emiroğlu , Director, Communicable Diseases and Health Security, WHO Regional Office for Europe Dr Masoud Dara , Coordinator, Communicable Diseases and Programme Manager, Joint Tuberculosis, HIV/AIDS and Hepatitis Programme, WHO Regional Office for Europe Professor Francis Drobniowski , ELI Core Group Chairman, Imperial College London
09:30–10:00	European TB Laboratory Initiative (ELI), overview of activities	Dr Soudeh Ehsani , ELI Core Group Secretariat, Joint Tuberculosis, HIV/AIDS and Hepatitis Programme, WHO Regional Office for Europe
Part 1. ELI TB and MDR-TB diagnostic algorithms		
10:00–11:00	ELI diagnostic algorithms for TB and MDR-TB in the WHO European Region	Dr Irina Felker , Executive director Novosibirsk TB Research Institute (NTRI)–WHO Collaborating Centre, Novosibirsk, Russian Federation
11:00–11:30	Coffee break	
11:30–12:15	Group work on case studies using the ELI diagnostic algorithms	Group work instructor: Dr Irina Felker Group work facilitators: Dr Gulmira Kalmambetova , Head of the National Reference Laboratory, Kyrgyzstan, ELI Core Group Member; Professor Sven Hoffner , Karolinska Institutet, Sweden, ELI Core Group Member
12:15–13:15	Lunch	
13:15–14:00	Group work presentation of 10 minutes and 5 minutes of discussion	Representatives of the working groups

Part 1. Keynote sessions		
14:00–14:30	Discrepancies between genotype and phenotype What is antimicrobial resistance and how do we define it (critical concentrations and minimum inhibitory concentrations)?	Dr Claudio Köser , College Research Associate, Wolfson College University of Cambridge, United Kingdom
14:30–15:00	Role of genome sequencing for TB and MDR-TB diagnosis	Dr Philip Supply , CNRS, Centre for Infection and Immunity of Lille, France
15:00–15:30	Panel discussion	Dr Philip Supply, Dr Claudio Köser, Dr Alena Skrahina, Dr Daniela Cirillo (via WebEx); Dr Masoud Dara, Professor Francis Drobniowski, Dr Soudeh Ehsani
15:30–16:00	Coffee break	
Part 2. TB laboratory biosafety		
16:00–16:30	ELI TB laboratory maintenance plan (LMP)	Dr Natalia Shubladze , ELI Core Group Member
16:30–17:30	TB laboratory biosafety	Dr Grigory Volchenkov , Vladimir Centre of Excellence for TB Infection Control, Vladimir, Russia Federation
17:30–20:00	Reception at UN City	

Programme 25 August

Part 3. MDR-TB diagnosis using WHO recommended MTBDRs/ VER 2		
Time	Topic	
09:00–09:15	Recapitulation of day 1 Presentation of the agenda	Dr Masoud Dara Professor Francis Drobniowski
09:15–09:30	MTBDRs/ VER 2 line probe assay to detect resistance to second-line anti-TB drugs (SL-LPA)	Dr Soudeh Ehsani
09:30–11:00	MTBDRs/ VER 2 toolkit for <ul style="list-style-type: none"> • result analysis and interpretation • result reporting • assay troubleshooting 	Dr Natalia Shubladze , ELI Core Group Member
11:00–11:15	Coffee break	
11:15–12:00	Group work (analysis of examples from the field)	Group work instructor: Dr Natalia Shubladze Group work facilitators: Professor Sven Hoffner, Dr Gulmira Kalmambetova, Dr Rasim Tahirli
12:00–12:45	Group work result presentation and discussion	Representatives of the working groups
12:45–13:00	Closing remarks	Dr Masoud Dara
13:00–14:00	Lunch	

Annex 3. Case studies

Case 1

Patient: 45-year-old female

Complaints: cough, fever up to 37.7°C in the last 8 weeks, weight loss of 5 kg

Radiography: infiltrative and destructive lesion of the lung tissue in the upper lobe and segment 6 of the right lung

Available laboratory diagnostic methods:

- microscopy
- Xpert MTB/RIF
- culture (solid media)
- BACTEC 960)⁸

What will you do?

Diagnostic steps:

- 1.
- 2.
- 3.
- 4.
- ...

If Xpert MTB/RIF positive for RR-TB?

Case 2

Patient: 35-year-old male

Complaints: cough, fever up to 38.7°C in the last 4 weeks, weight loss of 10 kg

Radiography: disseminative lesion of the lung tissue in right lung

Available laboratory diagnostic methods:

- microscopy
- culture (solid media)

What will you do?

Diagnostic steps:

- 1.
- 2.

⁸ BACTEC™ Mycobacteria Growth Indicator Tube 960 detects growth of mycobacteria in culture.

- 3.
- 4.
- ...

If Xpert MTB/RIF shows rifampicin sensitivity?

Case 3

Patient: 57-year-old female

Complaints: cough, fever up to 37.5°C in the last 4 weeks, weight loss of 4 kg

Radiography: disseminative and destructive lesion of the lung tissue in the upper and middle lobes of the right lung and segment 6 of the left lung

Available laboratory diagnostic methods:

- microscopy
- Xpert MTB/RIF
- FL-LPA
- culture (solid media)
- BACTEC 960

What will you do?

Diagnostic steps:

- 1.
- 2.
- 3.
- 4.
- ...

If Xpert MTB/RIF shows rifampicin sensitivity?

Case 4

Patient: 27-year-old male

Complaints: cough, fever up to 38.5°C in the last 10 weeks, weight loss of 6 kg

Radiography: disseminative and destructive lesion of the lung tissue in the upper and middle lobes of the right lung and segment 6 of the left lung

Available laboratory diagnostic methods:

- microscopy
- Xpert MTB/RIF

- FL-LPA, SL-LPA
- culture (solid media)
- BACTEC 960

What will you do?

Diagnostic steps:

- 1.
- 2.
- 3.
- 4.
- ...

If Xpert MTB/RIF positive for RR-TB?

Case 5

Patient: 43-year-old male

Complaints: cough, fever up to 38.0°C in the last 8 weeks, weight loss of 7 kg

Radiography: disseminative and destructive lesion of the lung tissue in the upper and middle lobes of the right lung

Diagnosis: disseminated tuberculosis of the upper and middle lobes of the right lung; positive for mycobacterium TB, resistance to streptomycin

Available laboratory diagnostic methods:

- microscopy
- Xpert MTB/RIF
- BACTEC 960
- culture (solid media)

What will you do?

1. Follow-up:
- 2.
- 3.
- ...

Case 6

Patient: 52-year-old female

Complaints: rare cough, fever up to 37.4°C in the last 6 weeks, weight loss of 3 kg

Radiography: multiple foci in the upper lobe of the left lung

Diagnosis: focal tuberculosis of the upper lobe of the left lung; positive for mycobacterium TB, drug susceptible

Available laboratory diagnostic methods:

- microscopy
- Xpert MTB/RIF
- FL-LPA SL-LPA
- culture (solid media)

What will you do?

1. Therapy?
2. Follow-up:
- 3.
- 4.
- ...

Case 7

Patient: 22-year-old female

Complaints: cough, fever up to 38.4°C in the last 6 weeks, weight loss of 5 kg

Radiography: infiltrative and destructive lesion of the lung tissue in the upper lobe and segment 6 of the right lung

Diagnosis: infiltrative tuberculosis of the upper lobe and segment 6 of the right lung; positive for mycobacterium TB, resistant to rifampicin

Available laboratory diagnostic methods:

- microscopy
- FL-LPA, SL-LPA
- BACTEC 960
- culture (solid media)

What will you do?

1. Follow-up (after culture conversion):
- 2.
- 3.
- 4.
- ...

Annex 4. Questionnaire on workshop content



