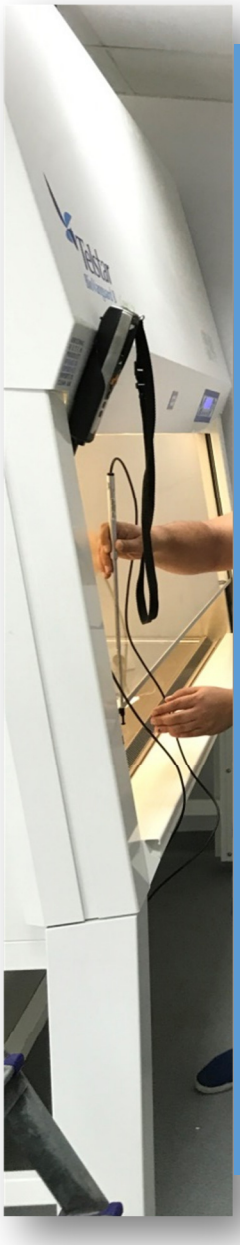
World Health Organization
REGIONAL OFFICE FOR Europe



Subregional Training on Tuberculosis Laboratory Biosafety Cabinet Maintenance

29 August–6 September 2017
Tbilisi, Georgia

Contents

Abbreviations

BSC	biological safety (biosafety) cabinet
BSL	biological safety (biosafety) level
NRL	National Reference Laboratory
NTP	National Tuberculosis Programme
PPE	personal protective equipment
TB	tuberculosis
UVGI	ultraviolet germicidal irradiation

Acknowledgements

The WHO Regional Office for Europe and the WHO Country Office in Georgia are grateful to the Ministry of Health of Georgia for hosting this subregional training course on biosafety cabinet maintenance for engineers and technicians.

Moreover, we would like to express our gratitude to the National Tuberculosis Programme and its manager, Professor Zaza Avaliani, and the National Reference Laboratory and its head, Dr Rusudan Aspindzelashvili, for their excellent collaboration and continuous support throughout the training.

This training was made possible with financial support from the United States Agency for International Development.

Executive summary

The aim of subregional training on tuberculosis laboratory biosafety cabinet maintenance is to enhance the capacity of qualified technicians and engineers to service biosafety cabinets (BSCs) in the region. Tuberculosis (TB), and particularly multidrug-resistant TB, are still a problematic issue in many countries of the WHO European Region. Laboratory diagnosis is of key importance for detection and treatment of TB. Properly equipped and maintained laboratories should be available for this purpose. Laboratory BSCs are intended to protect laboratory operators, products and the environment from harmful airborne agents. Their proper functioning and maintenance are key factors in attaining high safety levels and providing laboratory quality assurance. One of the major challenges that prevents the countries concerned from meeting this goal is a lack of qualified and trained technical personnel who can provide professional installation, field testing and maintenance of BSCs. In order to build national and regional capacity in TB laboratory BSC maintenance, the WHO Regional Office for Europe organized a subregional training session for technicians and engineers from selected countries (Georgia, Azerbaijan and Ukraine) in Tbilisi, Georgia, which ran from 29 August to 7 September 2017. This training was a follow-up to a similar session previously organized for Armenia, Belarus and the Republic of Moldova, which took place in Yerevan, Armenia, between 26 September and 5 October 2016.

Training background, scope and expected outcomes

In a clinical diagnostic laboratory, BSCs are key pieces of safety equipment. Their timely and correct functioning through qualified and prompt maintenance is of crucial importance if they are to fully perform their role as safety equipment. To date, most of the eastern European countries of the WHO European Region have had limited access to expertise to ensure timely preventive and corrective maintenance of their BSCs.

In order to build national and regional capacity in TB laboratory BSC maintenance, the WHO Regional Office for Europe organized two subregional training sessions for technicians and engineers from selected countries. The first was held in Yerevan, Armenia, between 26 September and 5 October 2016 for participants from Armenia, Belarus and Moldova; the second session was organized in Tbilisi, Georgia, for participants from Georgia, Azerbaijan and Ukraine, and ran between 29 August and 7 September 2017.

Target audience and process of candidate selection

The Ministries of Health of each of the invited countries were asked to officially nominate two engineers who fulfilled the following criteria:

- laboratory equipment technician or engineer with work experience related to laboratory maintenance of BSCs and/or ventilation systems;
- basic understanding of biological safety, general physics and filtration theory, as well as variables such as air velocity, flow rate, current, voltage and resistance;
- ability and commitment to work on the maintenance of BSCs in their own countries and elsewhere in the future;
- competence in English and/or Russian (with simultaneous translation and interpretation provided in both languages); and
- availability to undertake training during the proposed time period.

Participants received training first in theoretical aspects of TB laboratory BSC testing and maintenance following international standards; then in practical aspects which allowed hands-on experience and training.

After taking part in this training and successfully passing the final theoretical and practical examinations, the participants were expected to take responsibility for TB laboratory BSC maintenance in their own countries in the future.

Objective of the training

The objective of this training is to increase the national and regional capacity in BSC maintenance and to train qualified engineers based on international standards. Participants are expected to have responsibility for field testing and maintaining BSCs in the future. The training will allow BSC maintenance needs to be met more effectively; it will also improve sustainability and cost-efficiency in this important field within the WHO European Region.

Expected outputs and outcomes

It is expected that at least one trained engineer/technician from each of the participating countries will pass the final examinations on theoretical and practical aspects of BSC maintenance and certification. The expected outcome is that BSC maintenance will be sustainable and cost-efficient, allowing safe laboratory TB diagnosis.

Faculty members

Fig. 1 Faculty members (from left to right): Dr Paul Jensen, Dr Soudeh Ehsani, Mr Jörg Weber, Ms Svenja Schneider, Dr Grigory Volchenkov.

The faculty was composed of international experts in the field of TB biosafety and infection control: Dr Paul Jensen, Lead for TB Infection Control and Biosafety, CDC, Atlanta, USA; Dr Grigory Volchenkov, infection control specialist, Vladimir Regional TB Control Centre, Russian Federation; Ms Svenja Schneider, engineer at one of the main BSC third-party certifiers; and Mr Jörg Weber, an engineer with extensive experience in BSC maintenance in the eastern part of the WHO European Region and elsewhere.

The training session was organized and coordinated by Dr Soudeh Ehsani, European TB Laboratory Initiative (ELI) Secretariat and focal point for TB and MDR-TB Laboratory Diagnostics, Biosafety and Infection Prevention Control in the WHO European Region.

THEORETICAL PART

Day 1

Welcome and introduction

Fig. 2 Professor Zaza Avaliani, manager of the National Tuberculosis Programme (NTP), and Dr Rusudan Aspindzelashvili, head of the National Reference Laboratory (NRL), welcome the participants.

Professor Zaza Avaliani, manager of the Georgian NTP, addressed the participants on behalf of the staff of the National TB Centre. He expressed his gratitude to the WHO Regional Office for Europe, which was responsible for organizing the training course involving participants from Georgia, Ukraine and Azerbaijan; the aim of the course was to enhance the regional and national capacity of technicians and engineers in the technical maintenance of BSCs. He welcomed those responsible for the training and stressed that it was a great honour to have Georgia host the training. Professor Avaliani pointed out that, although the number of TB cases in Georgia was falling, the spread of multidrug-resistant TB was still a problem. Culture tests, he explained, were conducted in the referral laboratory, which meant that laboratory staff had to deal with living TB cultures on a daily basis, and hence it was essential that strict biosafety standards were observed. Noting that laboratory biosafety played an important role in clinical diagnostics, Professor Avaliani observed that Georgia suffered from a shortage of professional technical staff and that the training session was a good opportunity to build capacity in the area. He also spoke of the advantages of having a new laboratory in Georgia, which had been equipped with the assistance of the Global Fund to Fight AIDS, Tuberculosis and Malaria.

Dr Soudeh Ehsani, of the WHO Regional Office for Europe, was next to address the participants with a welcome speech. Dr Ehsani thanked the NTP manager for his support in various projects and welcomed all the participants on behalf of the WHO Regional Office. She pointed out that the shortage of staff qualified to maintain BSCs – the problem that the training session was intended to address – represented a major challenge in the Eastern part of the European Region. Dr Ehsani mentioned an earlier successful training session, in September 2016, which had involved the participation of engineers and technicians from Armenia (the host country), the Republic of Moldova and Belarus. She thanked all the Ministries of Health that had nominated participants for the current training and, in particular, expressed her thanks to the NRL for hosting the training in its new laboratory premises. Dr Ehsani then proceeded to introduce the faculty members and the training agenda. This comprised theoretical and practical parts, each ending with an exam: the first covering the theoretical knowledge and skills gained by the participants upon completion of the training; the second covering the practical knowledge and skills they had acquired.

Biosafety

Ms Svenja Schneider, spoke of the importance of having a proper legal basis for certification of BSCs. By way of example, she described the legal structure as it is organized in Germany and stated that, while the constitution is paramount in the legislative framework of any country, it does not contain any provisions on BSCs (though in Germany there are relevant laws and regulations that contain provisions on BSCs). Ms Schneider continued with an explanation of risk assessment, which requires an understanding of laboratory procedures in order to determine appropriate protective measures. She also mentioned the importance of documentation.

The session then moved on to consider how a laboratory's biosafety level (BSL) is determined. A particular BSL is assigned to a laboratory on the basis of the biological agents it handles and its work practices, safety equipment and facilities. There are always three things that require protection: the worker, the environment and the product; consequently, the appropriate protective measures depend on the priority given to these items. Next, Ms Schneider touched upon the risk groups of biological agents, explaining how agents are classed at four different levels; she explained how the hazard increases from BSL 1 to 4 and described the safety equipment, work practices and facilities appropriate for each level. As it happened, none of the countries represented at the training session had a BSL-4 laboratory, and only Ukraine had a BSL-3 laboratory (for the veterinary sector).

The presentation continued with a discussion of personal protective equipment (PPE) – protective clothes, gloves and respirators – in the laboratory; it was stressed that a proper risk assessment was required to select appropriate PPE. Ms Schneider then gave definitions of disinfection and sterilization and explained the principles of handling disinfectants. At the end of the presentation, she gave a classification of fungi, viruses, parasites and bacteria, explaining that classifications differed from country to country.

During the discussion, Dr Paul Jensen (of CDC, Atlanta) asked trainees to consider, and be aware of, applicable national standards, norms and regulations with respect both to biological safety in general and to BSC certification, testing and maintenance. During the discussion with participants, it turned out that, for the most part, there was a lack of such documents in their countries; they explained that relevant European legislation and WHO regulations and manuals for TB laboratories were generally used instead. The participants complained that there was a shortage of certified specialists in their countries who could validate BSCs; they stressed the importance of training in obtaining the relevant qualifications.

Proceeding with the presentation, Ms Schneider then asked the trainees whether it was the pathogen itself, the classification of the pathogen, or how the pathogen was handled that influenced the risk level. One of the participants explained that manipulations of a serious pathogen without the necessary precautions represented a high risk, which was reduced by use of PPE and proper equipment. Another participant added that in their respective country, in BSL-1 laboratories, where only microscopy was done, there were no BSCs, while in BSL-2 and BSL-3 laboratories, where culture was done, BSCs were mandatory to protect both the lab worker and the product. Dr Grigory Volchenkov then pointed out that many factors had to be taken into account when assessing risk. Dr Soudeh Ehsani commented that laboratories worked with different pathogens with different transmission routes, so appropriate protection measures should reflect these differences. Ms Schneider summarised that, when carrying out a risk assessment, personnel should gather relevant information and then brainstorm the issue to determine the level of risk and hence the biosafety measures required. On the one hand, there were laboratory personnel who knew about internal procedures; on the other hand, there were service engineers, who had never worked with biological agents. For this reason, the BSC maintenance professional (certifier) should understand the basics of biosafety, collect and analyse relevant laboratory information, and take appropriate precautions on the basis of a risk assessment.

The question whether decontamination is necessary when maintaining BSCs was raised by the trainees. At the end of the discussion, it was decided that the need for decontamination, and the frequency with which it was carried out, depended on the kind of procedures performed in

the BSC and the kind of maintenance routine in place. At the end of the session, the participants practised disinfecting their hands using a special fluorescent liquid and an ultraviolet (UV) spotlight.

Biological safety cabinets (BSCs)

Ms Schneider gave an introductory presentation on BSCs, explaining the background and the need for training on BSC maintenance. She stressed the importance of performing field testing in the proper way following the respective Standards. She pointed out three main factors with respect to BSC safety: independent certification of BSCs, qualified handling, and qualified service. She then outlined third-party certification that employed the EN 12469 or NSF/ANSI 49 standards. A list of the most commonly used standards was introduced:

- European Union – EN 12469
- Russian Federation – ГОСТ Р ЕН 12469-2010 (GOST R EN 12469-2010)
- USA – NSF/ANSI 49
- Japan – JIS K 3800
- China – SFDA YY 0569.

Prior to EN standards (2000), the following standards were in place:

- DIN 12950 Teil 10
- NF X44-201
- BS 5726 Parts 1–4.

Ms Schneider mentioned that the Russian standard (GOST) was translated from, and complies with, European standard EN 12469. She indicated that almost all the information relevant to the various standards was available on their respective web pages. The definition of BSCs was given to the participants. Then an overview of BSC classification was presented. Ms Schneider outlined the classes according to EN 12469:2000 – Class I, Class II, Class III; and according to NSF/ANSI 49 – Class I, Class II (Types A1, A2, B1, B2) and Class III. She added that for NSF there would be a new Class II Type C cabinet, with additional benefits compared to Class B cabinets. Class II Type A2 (NSF/ANSI 49) and Class II (EN 12469) cabinets were recommended by WHO for TB laboratories. She then gave a detailed overview of cabinets of each class: Class I cabinets ensure only personal protection and give no protection either to the product or to the environment; Class II (EN 12469) cabinets ensure personal protection and protection of the product, including protection against cross-contamination, and environment protection. Her

recommendation was to follow the manufacturer's instructions when doing field testing of cabinets.

The presentation then moved on to Dr Paul Jensen's review of Class II Type A1 BSCs according to NSF/ANSI 49. Dr Jensen started with a definition of "plenum", explaining that the plenum was a restricted space in the rear part of the cabinet, and proceeded with a description of an A1 cabinet. He mentioned that A1 cabinets were no longer produced but were still in use. He then gave an overview of cabinets of Class II Types B1 and B2.

Ms Schneider presented a table which included test methods for type testing, installation testing and routine maintenance testing for Class I and II BSCs. She then touched on the operating parameters for Class II Type A2 BSCs and explained that containment was achieved by inflow and downflow that created a barrier to the front opening; it was very important to have the correct barrier, so the manufacturer's instructions should be followed. The integrity of the filter, as well as the tightness of gaskets, was also important for the achievement of containment. She then elaborated on ducting cabinets, emphasizing that WHO does not recommend using models that require hard ducting (B1 and B2). Ducting of Class II A2 cabinets could be considered only using a thimble system. She explained that safety and effectiveness of BSC ducting also depend on the parameters of the laboratory ventilation system.

During the discussion, Dr Jensen notified participants to be watchful with their national standard, as there were statements in GOST as an example that were not 100% accurate. In addition, Dr Grigory Volchenkov was curious whether EU countries manufactured cabinets of Class II B; Ms Schneider replied that some manufacturers were considering manufacturing these cabinets, but, as a rule, Class II A2 cabinets were preferred.

In the course of the discussion, Dr Volchenkov suggested that certain terminology should be clarified. He explained that the upper filter was called the "exhaust filter", as its function was to filter contaminated air and expel it into the exhaust system; the lower filter was called the "downstream filter". Repeating the definition of "plenum", Dr Volchenkov went on to explain why the plenum should not be under positive pressure and how it was dangerous for it to become so. One of the participants supposed that, in the event of an accident, there would be an invisible leakage of aerosol from the plenum into the room. Thus, Class II Type A1 cabinets did not have an unprotected plenum, as it was under positive pressure. Dr Volchenkov also added that, to his knowledge, there were no Class II Type B cabinets in the countries of the former Soviet Union. Then he explained that the space behind the glass is called the "working area", where all manipulations are performed – it is sterile and situated in a sterile

downstream, as the lower filter supplies it with pure filtered air by means of a laminar flow. In addition, there was a physical barrier in the form of a protective window.

Then the discussion moved on to the issue of tissue getting caught in the filters. One of the participants asked whether the lower prefilter could catch microparticles or chemical agents, to which Ms Schneider replied that it was the same as the other filters. Then the question of the necessity of upper filters arose; Ms Schneider replied that manufacturers asked the same question, but it was a matter of ensuring additional protection.

Next, the issue of ducting was discussed, which led to a number of conclusions. Direct ducting of Type A cabinets is not permitted – they must be exhausted only through a properly designed and fitted canopy exhaust system. Canopy-connected Type A cabinets require a consistent, low static pressure. While a dedicated exhaust system is preferred, they may share a common exhaust system with other exhausted laboratory devices, provided they are properly balanced. Type B BSCs require a higher static pressure which must increase as their exhaust filters load. They must be on a dedicated exhaust system, and not ganged with other varieties of Type B BSCs, or other exhausted laboratory devices requiring a lower static pressure (such as fume hoods and canopy-connected BSCs). As a rule, it is not accepted practice to allow a BSC to positively pressurize an exhaust duct in normal operation. For a Type A BSC fitted with a properly designed canopy connection, reduction or elimination of exhaust air should not significantly affect the airflow patterns within the BSC; personnel and product protection of the BSC should remain unchanged. Chemical vapours generated in the BSC would be exhausted into the laboratory via the exhaust filter if the cabinet was not properly ducted to the ventilation system.

Test types and testing methods

Ms Schneider gave a presentation on BSC testing methodology. She talked about test types and presented a table with a clear division of responsibilities between manufacturer and maintenance provider. She then continued with a brief overview of test methods, including type testing, factory acceptance test, installation testing and routine testing; she defined each method and indicated who was responsible for carrying it out. Next, Ms Schneider explained that everything should be checked on the basis of the cabinet manufacturer's specifications. She concluded by stressing the importance of documenting all test results, so that a full history was available. She told the trainees how important it was to carry out a risk assessment before maintenance; this should cover general risk assessment; identification of the types of work performed in the laboratory; an assessment of biological, chemical, mechanical and electrical risks; and an inspection of the ventilation system. Then she talked about the importance of

annual certification of BSCs, as well as their certification after any repair, move or relocation, which should be carried out by personnel trained in BSC testing and maintenance.

Ms Schneider presented the equipment required for certification of a cabinet:

- anemometer
- aerosol generator
- photometer or particle counter
- diluter
- smoke tubes
- measurement device for electrics
- evaporator for BSC decontamination.

Ms Schneider went on to point out the necessity of visual inspection and stressed that no unnecessary items should be stored in or on the cabinet. She then gave examples of BSC assessment for leak tightness of carcass, talked about measurement of inflow and downflow velocities, and explained filter testing methods (filter integrity, gasket leakage), emphasizing that each filter should be tested individually. She stressed the importance of smoke testing for visualization of flow direction and possible turbulence, as well as disturbances from room air currents and drafts. She then moved on to electrical safety testing and concluded with a reminder that all results should be fully documented. Specifically, the report should include all data relating to the equipment tested; measurement and test programme with conclusion, including measurement results, as well as measurement grids and limit values; the name of the service technician; instruments used and calibration data; an inventory list and room plan; and overall conclusion.

The remaining part of the presentation was dedicated to calibration of testing equipment and its importance. Ms Schneider emphasized that it was necessary to calibrate testing equipment at least once a year, with particular attention paid to selecting a suitable calibration laboratory and correct calibration range. The calibration laboratory should preferably be accredited according to ISO 17025 (or similar). She finished this presentation with tables showing calibration ranges.

Fig. 3. Dr Grigory Volchenkov.

After Ms Schneider's presentation, Dr Volchenkov made a brief presentation on the use of respirators for people working in conditions of high risk of airborne infections. Dr Volchenkov pointed out that there is a big difference between a respirator and a mask; he stressed that respirators should always be used in high-risk settings and that each laboratory worker should know the particular size and model that fits them, as there are many different models on the market (including fake ones that represent a risk to users). Dr Volchenkov then moved on to standard EN 149, which stipulates initial penetration (filtration efficiency of the material), though it should be remembered that no respirator guarantees 100% protection. One of the most important points about respirators is that incorrect use can increase leakage: to be protected, it is not enough to use the correct model of respirator – one should know how it is worn and test it each time before putting it on. In conditions of high risk of infection, respirators of type/class N95, N99, FFP2 or FFP3 should be used. Dr Volchenkov mentioned a quick fit check test to detect rapid leakage in a respirator. Put on the respirator according to the instructions attached to the package (if you get a respirator without usage instructions and/or standard and model signage, it is probably a fake); then take two or three forced inhalations and exhalations: if air leaks through the gap between skin and respirator, you should check it and fix the leakage source before starting work in it. Dr Volchenkov listed the main information a respirator should include: name of the standard, protection class, name of manufacturer, and model.

Next, Dr Volchenkov moved on to the fit test, which can be performed by means of a special testing set. Ideally, a coordinator with special equipment should be designated to assist in choosing the correct size and model of respirator for all individuals working in settings where there is a high risk of airborne infection. As the shape and size of a person's face may change over time, it is worth spending 10 minutes a year to have a respirator fit test, to make sure a respirator provides the greatest possible protection.

During the discussion, a question arose whether certification of cabinets should be conducted after their acquisition. Ms Schneider assumed that there was some confusion over terminology and explained that, after purchase of a cabinet, a field test was done on site. Then Dr Volchenkov gave the Russian equivalent of the term "field test" and explained that, after a new cabinet had been delivered to a laboratory and installed, it should be tested on site and then subjected to routine testing annually, which was a field test as an essential part of maintenance. During the discussion of measurement of inflow and downflow velocity, a question arose whether there was a difference in standards with respect to measurement of downward flow.

When calibration Ms Schneider mentioned that for anemometers it was not difficult to find an accredited laboratory, but for photometers it was quite challenging to find an accredited lab in Europe. Dr Volchenkov added that there were problems in the Russian Federation regarding provision of metrological services. The discussion then moved on to calibration of particle counters and diluters. Ms Schneider explained that both particle counters and diluters required calibration. She explained that one way of checking the accuracy of calibration was to compare data from different anemometers, but one had to be sure that one of them was correctly calibrated. Dr Jensen suggested that a good way of checking proper calibration was to take data of a response curve prior to sending equipment for calibration; data after calibration could then be recorded, which would provide a picture after a period of a year.

Day 2

Protection test methods

Ms Schneider spoke about microbiological testing according to EN 12469 and presented a table of test methods for type testing, installation testing and routine maintenance testing for Class I and Class II BSCs. She explained that, for containment of Class II BSCs, the following tests were conducted: filter test (EN 12469, Annex D, VDI 2083 BI. 3); leak tightness of carcass (EN 12469, Annex B); and microbiological test (EN 12469, Annex C) for personal protection or the retaining capacity at the work opening, for product protection and cross-contamination. Ms Schneider stressed that testing of the protective functions at a BSC specific set point in relation to the flow rate should be performed in accordance with the manufacturer's instructions.

Ms Schneider then spoke about reference methods in microbiological testing as described in the following standards:

- USA – NSF/ANSI 49
- Europe – EN 12469, DIN 12980
- Russian Federation – GOST R EN 12469
- Japan – JIS K 3800
- China SFDA YY 0569.

Ms Schneider continued by giving definitions of types of protection according to EN 12469. She explained that personal protection was the ability of a BSC to protect the user and the surrounding environment from generally particulate, biological and/or chemical substances. Product protection was the ability of a BSC to protect a product used within the workspace from particulate, biological and/or chemical matter present in the surroundings or the environment. Cross-contamination occurred when a BSC failed to protect a product used within the workspace from particulate, biological and/or chemical matter from other materials or products inside the BSC. She then spoke about each type of protection in detail and presented relevant pictures, diagrams and equations.

Ms Schneider then spoke about the advantages and disadvantages of reduction of airflows. The advantages of reduction appear to be as follows:

- sound pressure is lowered;
- work surface vibration is generally lowered;
- filter life is extended;
- energy consumption may be decreased.

And the following are the disadvantages:

- the safety functions are decreased – or may even disappear altogether;
- ability to compensate for interference in the surroundings is decreased.

During the discussion, the participants wanted to know whether the protection tests Ms Schneider spoke about should be performed once a year. She explained that, as a rule, these tests were performed by the certification company – they were presented in the training to raise participants' awareness of what a certification company usually does. Concerning the activities of certification companies, a question arose whether laboratories had to apply to a certification company annually for performance of certification. Ms Schneider replied that it was the responsibility of engineers to check the flows in cabinets annually – specifically, to check that the flows met the manufacturer's requirements, and if they did, that meant that the cabinet was safe.

Then Dr Volchenkov explained one of the tests conducted by certified laboratories: they took one sample of a cabinet from the manufacturer – a new model or an old one for recertification – to certify the model itself; then, after each five-year period, the manufacturer would submit the cabinet for similar certification, while the model was certified as compliant with EN 12469. Thus, each particular model of cabinet received a certificate of compliance with the EN standard. It was also stated that the field test should be performed according to the standard which the manufacturer had applied during certification. Ms Schneider added that, in Germany, only the EN standard was applied, and in the USA, only the NSF standard, so – in that respect – the application of standards did not require a great deal of thought.

Next, several scenarios and threats involving low/high inward/downward flow were discussed. The first scenario was when the downward flow was low/weak and the inward velocity high: in this case, the protective capacity of the air curtain was lost and contaminated air could enter the work area. The next scenario was when both downward flow and inward flow were weak: the product was contaminated, and there was a risk that both product and operator could lose protection. The third scenario was when there was a strong downward flow and a weak inward flow: in this case, the laboratory worker was not protected, but the product was. The best option was to follow the manufacturer's specifications.

Then there was a question from one of the participants on how the inflow velocity could be decreased without regulation of a fan? Ms Schneider explained that inflow velocity should not be decreased, as tests should be performed in accordance with the manufacturer's specifications. Decrease of inflow velocity could lead to contamination of the product.

During a discussion of certification of cabinets by third parties, it was asked what standards should be used during certification if a laboratory had cabinets made by different manufacturers. The answer was that the standard used for the field-testing would need to be in accordance with the standard to which the cabinet was built.

Presentation: impact of ventilation

Ms Schneider gave an overview of a laboratory, focusing on the ventilation system, and explained the factors affecting BSC airflows. She mentioned that a general rule of thumb was used to determine the existing ambient conditions, and as a result, the protective functions of a safety cabinet were not always achieved. Then she touched on possible types of ventilation for TB laboratories, according to WHO recommendations. These were:

- unidirectional airflow with 6–12 air changes per hour;
- BSC ducted with a thimble connection;
- a small gap (usually 5 cm) maintained between the thimble and the cabinet's exhaust opening;
- the ventilation system for the laboratory designed by a qualified specialist engineer.

Fig. 4. A training participant uses a vane anemometer to measure the upper-room ventilation system airflow.

In her presentation Ms Schneider explained how calculation of air changes per hour should be performed – in particular, how to determine the volume of the laboratory space and the amount of air exhausted from each cabinet. Then she spoke about the location of BSCs in the laboratory and presented a recommended setup; she also mentioned the impact of ventilation systems on BSCs.

During the discussion, the participants asked whether there were any national regulations in Germany dealing with ventilation systems; Ms Schneider replied that Germany had

recommendations, rather than regulations. There was much discussion of laboratory ventilation systems and a range of different opinions were expressed.

Presentation: decontamination

Ms Schneider gave a presentation on BSC decontamination and explained the importance of risk assessment. Such assessment consists of the following:

- identification of biological agents
- assignment of activity to a biosafety level (BSL)
- definition of protective measures
- drawing up a list of all biological agents
- documentation of hazards.

Ms Schneider underlined the possible dangers of formaldehyde fumigation and said that fumigation – if it was necessary at all – should be carried out only by a relevantly qualified specialist. She then explained that formaldehyde vapour was usually used for fumigation and outlined the hazard levels associated with it. Next, she dealt with personnel safety when formaldehyde was used and the necessity of having personal protective equipment (PPE) at hand; she also gave recommendations regarding proper sealing of cabinets and actions following fumigation. The procedure for preparing cabinets and the laboratory for fumigation with formaldehyde was described, as were prevention and response measures. Then Ms Schneider presented various recently developed decontamination procedures that do not involve formaldehyde.

During the discussion, a TÜV BSC maintenance certificate was reviewed in detail. Then examples of other certificates were presented and discussed.

The use of formaldehyde caused much discussion, in which the participants expressed conflicting opinions regarding its use and hazardous nature. Examples of improper use of formaldehyde were given, leading to the conclusion that it should only be used with caution. The participants then discussed how to seal a cabinet when preparing for formaldehyde treatment, stressing that insulation was an important part of fumigation; the concentration in the room should be kept as low as possible and the room should not be contaminated. It was pointed out that gas should circulate through the filter. It was discussed that a liquid solution could be used, which could be brought to a gaseous state by boiling. Some of the participants appeared to prefer formalin, but it was agreed that both methods yielded the same results. It

was mentioned that in some countries formaldehyde was completely forbidden and that there were alternatives, like decontamination with H_2O_2, available on the market.

Airflow measurement

Fig. 5. Ms Svenja Schneider.

Ms Schneider gave a presentation on airflow measurement and explained that there were flow processes in nature, as well as technical flow processes. She then spoke about pressure, density and velocity and how to measure them. She then considered two types of flow – laminar flow and turbulent flow – and presented equations to calculate them.

Ms Schneider touched on measurement of airflow rate and stressed the importance of accurate measurement. The general rule is first to analyse the problem, then make measurements. Various kinds of error must be prevented or minimized, including errors of the measuring instruments, errors of adjustment and errors in data recording. Instruments should be calibrated regularly (once a year). Data control is also important; always expect inaccuracies, and never blindly believe your data.

Ms Schneider presented instruments used for the measurement of air velocity – Prandtl (Pitot) tube, rotating vane anemometer, thermistor, hot-wire anemometer, laser Doppler anemometer – and discussed their advantages and disadvantages. Particular attention was paid to the DIM method of BSC flow rate measurement – “direct inflow measuring”. She explained the exhaust method of measuring inflow velocity and gave an example to show how it was calculated. She then explained downflow velocity measurement and spoke about corrections of

pressure and temperature in the event of significant deviations from the standard values. Ms Schneider stressed the importance of proper documentation of measurements, whatever type of measurement was chosen. She then moved on to flow visualization, which is used only for qualitative assessment of BSC flows; she spoke about its advantages and disadvantages, and also explained the measurement procedure using photos.

Fig. 6. Practical airflow measurement and explanations at the BSC.

During the discussion, the participants were asked to explain why the opening to the work area was partly closed when airflow was measured. If it is fully open, no protection is provided; protection is ensured within 20 cm, so it is important to set the opening in its operational state. It is essential to know the measurement protocol recommended by the BSC manufacturer and to understand its approach. The participants then discussed correction of flow measurement – pressure and temperature correction – noting that, in the event of significant deviations from the standard values, some correction might be necessary. The participants stressed that there were many factors influencing the method of measurement, so a choice that was appropriate to the circumstances should be made. Ms Schneider recommended choosing the method recommended by the manufacturer and following the instruction manual.

Day 3

Presentation: filters

Ms Schneider gave a presentation on filters. She presented the HEPA (high efficiency particulate air) filter and indicated that its purpose was to catch small particles. She then explained the structure of the HEPA filter and the way it performed filtration, and gave a summary of filtration factors, which were:

- particle size
- particle mass
- airflow velocity.

Ms Schneider also explained that the efficiency of HEPA filters depended on their classification, which was the proportion/percentage of particles caught by the filter. She noted that the HEPA filter leak test was described in several standards and guidelines:

- EN 1822-1
- ISO-14644-3
- VDI 2083 Part 3
- IEST-RP-CC006.2.

The principle of choosing appropriate filters was then discussed. This led to the conclusion that, when choosing a filter, it was very important to know what kind of microbes a laboratory was going to work with.

Next, Ms Schneider dealt with the equipment used to test filters. She explained how photometers and particle counters worked, noting the advantages and disadvantages of each. She also stressed the importance of calibrating test equipment, stating that the time between calibrations should be no more than 12 months.

Ms Schneider then moved on to filter tests and stressed the importance of not damaging the HEPA filter. She described in detail the procedure for testing with a photometer, listing all the steps of the measurement and giving relevant formulas. The same was done for testing with a particle counter. She then outlined the leakage test, explaining how the filter was scanned in order to identify any leakage. She also mentioned the problems that might arise during scanning and explained how to prevent them. At the end of the presentation, she once again emphasized the advantages and disadvantages of photometer and particle counter.

During the discussion, Ms Schneider asked if anyone had previously tested filters for leakage, as it appeared that some of the participants already had experience in testing filters with a particle counter. The question arose whether a particle counter or a photometer was better for testing, to which Ms Schneider replied that each had its advantages and disadvantages and that it depended on the BSC testing standard and personal preference. There was some discussion of filter warranties; it turned out that filters have no warranty, and no one could say how long they might last – it depended on the cleanliness of the ambient air, though mechanical damage could reduce a filter's durability.

Next, calibration of equipment was discussed. It was stated that all equipment for filter testing, with the exception of aerosol generators, should be calibrated. There was much discussion of the need to remove the metal grid when performing measurements. The opinions of participants on this issue were divided, but it was concluded that the grid should be removed as per the manufacturer's manual. The participants also discussed the need to change filters, and it was suggested that the filter should be changed when it was damaged, failed a filter test, had problems with uniformity of flows, or was clogged. In the end, the participants concluded that an individual risk assessment should be performed for each cabinet and that an annual filter change was not required.

The participants then discussed the possibility of repairing a filter if leakage was detected. There were different opinions, but the participants, together with the facilitator, identified cases when the filter could be repaired (provided the repair was agreed with the end user) and then went through the steps of the repair.

BSC testing types in international standards

Fig. 7. Dr Paul Jensen explaining different types of BSC thimble ducts.

Dr Paul Jensen gave a presentation on different types of BSC testing and responsible parties. He stated that there were three main categories of testing: cabinet-specific or type testing; installation testing; and routine maintenance or annual field testing. He explained that the manufacturer performed type testing to prove that a given unit complied with a particular standard: by testing one model or one cabinet, the manufacturer could confirm that the model of the cabinet complied with the standard. Dr Jensen went on to explain that there was a second category of type testing, which was third-party testing. In this case also, one model was tested, and then a report was issued, on the basis of the third-party report, that contained model, serial number and other required information. Dr Jensen pointed out that a third-party certificate was applicable not only in the case of cabinets, but also in the case of other equipment, such as ventilation. Next, he explained that there was one further type of testing: factory-acceptance testing, in which a factory would do a series of tests on each cabinet prior to shipment out of the factory, as a result of which a factory-accepted test report was compiled, which contained the test types and steps that had been performed. Dr Jensen then spoke about installation testing, which took place after installation of a cabinet and assured the owner that it worked properly. Finally, Dr Jensen came to routine maintenance testing, which combined maintenance and testing; its aim was to make sure that the cabinet provided personal protection, product protection and environment protection.

During the discussion, the participants tried to work out which type of testing – third-party testing or self-declaration – was preferable to ensure that a cabinet met international standards. Some participants preferred third-party certificates; others said that both certificates were required. Dr Volchenkov expressed his opinion that manufacturers were commercial entities interested in increasing their sales, so independent testing should always be conducted. Participants wanted to know if third-party certification was mandatory or not: it was recommended for good customer assurance, though some cabinets that failed third-party testing were nevertheless of high quality. After Dr Jensen had spoken about installation testing, a question arose as to who was responsible for this kind of testing: the responsibility depends on the wording of the tender – it may be the manufacturer of the BSC or a third party, or even the owner. Consequently, if there is no provision concerning installation testing in the tender, the responsibility lies with the laboratory.

When asked what type of BSC testing was most important, the participants answered that all were equally important.

Decontamination and disposal of filters

Ms Schneider gave a presentation on decontamination, safe filter change and disposal. She explained when decontamination was necessary and the basics of the procedure. She then listed the main points to be considered before starting work, which included (among other things) the types of microorganism being manipulated in the cabinet, effective dust protection, and kinds of disturbance. She explained the procedure for filter disassembly in detail. She showed photos to illustrate her points, while emphasizing that PPE must be worn throughout the whole procedure.

Additional testing

Ms Schneider gave a presentation on additional BSC testing, which included testing of lighting; measurement of sound pressure level, vibration and temperature; and checking for leakage of carcass, cleanability, ergonomics and UV light. She listed procedures for each type of test/check.

PRACTICAL PART

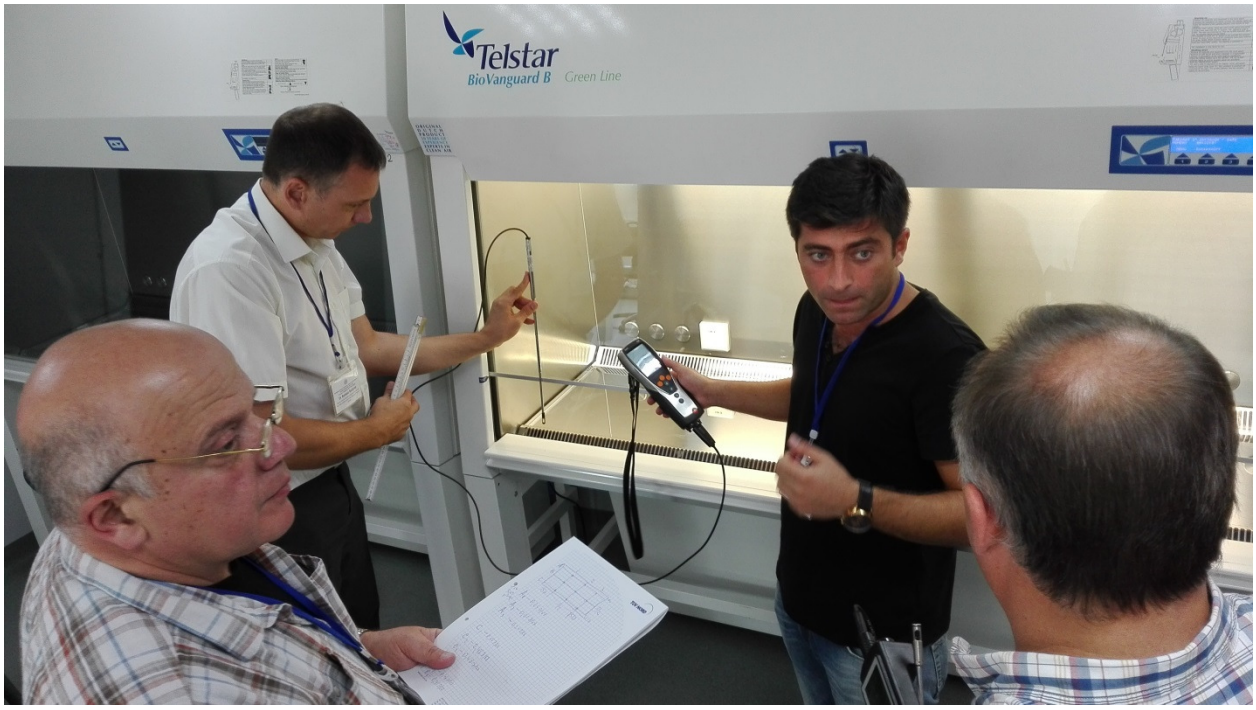


Fig. 8. Inflow air measurement by training participants using an anemometer.

Day 1

Measurement instruments – airflow visualization – airflow measurement and calculation – BSC airflow adjustment

The practical part of the training course was conducted in Georgia's new TB National Reference Laboratory (NRL), which was in the commissioning and acceptance period at the time of the training. Some practical sessions took place in the older operational NRL building. After the introduction, instructions and demonstration of each BSC testing procedure, every participant practised individually on a functional BSC using all necessary instruments and tools required for training purposes.

The practical sessions started with an introduction to BSC airflow measurement techniques, which were then demonstrated in the laboratory. The practical aspects of handling hot-wire anemometers, vane anemometers, aerosol generators, diluters, photometers, particle counters, smoke tubes and UV-C radiometers were discussed. Mr Jörg Weber demonstrated the particle counter and photometer, and techniques of HEPA filter testing. Generation of standard challenge aerosol was discussed and practised for downflow and exhaust filter testing. Measurements with particle counters and photometers were demonstrated to the participants;

the differences between them were identified and discussed. Flow rate, air velocity, air changes per hour in the high-risk area were measured by the participants using an anemometer. A special session was dedicated to direct and indirect BSC airflow measurements. Ducting of the BSC to exhaust was assessed and discussed in detail, including design, flow rate calculation, sizing, installation, commissioning and monitoring. Performance of a smoke test for demonstration and field test certification purposes was demonstrated and subsequently conducted by each participant. Participants practised BSC airflow assessment by smoke to verify flow directions and to assess air velocities, quality of laminar flow from downflow filter, effectiveness and safety of exhaust thimble connection, influence of mechanical ventilation and other external factors on BSC performance in terms of operator, product and environment protection.

At the end of the day, BSC adjustment based on field test findings was discussed. The logic and methodology of certifier actions in the event of detected inflow or downflow deviations were introduced and discussed in detail.

Day 2

UVGI: theory, measurement, design, installation and maintenance – laboratory ventilation system assessment – filter testing

Fig. 9. Downflow filter test using a particle counter, performed by one of the training participants.

The second day of the practical part of the training started with an introduction to ultraviolet germicidal irradiation (UVGI), the physical and biological properties of UV radiation, and the

principles of its measurement by a UV-C radiometer (irradiance in microwatts (µW)/cm^2). UVGI irradiance measurement, dose and exposure calculations were practised. The principles of upper-room UVGI design, installation, commissioning and maintenance were presented.

Laboratory ventilation system design was presented and compared to the actual installed ventilation system. Participants evaluated the actual installation of a mechanical ventilation system in the high-risk area of the laboratory. Necessary exhaust and air supply flow rates, as well as differential pressures required for a given number of BSCs, were discussed.

After participants' reports on BSC airflow findings during the previous day, actual air down- and inflow velocities in the BSCs were compared to the manufacturers' set range of velocities, which requires adjustment. Values were measured with different anemometers and recorded by the participants; the differences, and the underlying reasons for these differences, were then discussed.

Downflow and exhaust filter testing using a particle counter and a photometer was demonstrated by the trainers and conducted by participants in two separate groups. Participants learned and practised the methodology of standard challenge aerosol generation, calculation of filter penetration, scanning of filter surface by probe, and detection of filter leakage. The principles of repairing leaking HEPA filter defects were discussed in theory.

Day 3

Documentation and reporting – prefilter testing – electrical safety – BSC decontamination

The practical documentation and reporting session included demonstration and discussion of BSC field testing report templates from different companies.

After demonstration of the first/prefilter testing procedure with a particle counter and photometer, the participants practised it individually on a functional BSC.

Mr Jörg Weber introduced and demonstrated BSC electrical safety evaluation as part of routine maintenance testing.

The practical session on BSC decontamination covered safety precautions, including PPE use, calculation of decontaminant dose and exposure, demonstration and practice of related equipment (formalin evaporator). This was followed by hands-on decontamination performance practice by the participants (for training purposes, they used water instead of actual decontamination chemicals – formalin and ammonia).

Day 4

Final practical exam – panel discussion – closing remarks

On the last day of the practical part of the training, each participant individually performed BSC field testing and filled in the test report under supervision of a dedicated instructor. As a practical examination, participants performed a full visualization test of BSC airflow patterns using smoke tubes; measured and calculated volumetric airflow rates; carried out downflow, exhaust and prefilter testing; and conducted a site installation assessment. Each participant independently used smoke tubes, hot-wire anemometer, aerosol generator, diluter, particle counter and photometer for BSC testing; and filled in an individual test report, including general conclusions and recommendations based on relevant findings. All six participants successfully passed the final practical exam.

A panel discussion following the exam was dedicated to available international and national guidelines and norms on BCS certification and maintenance, as well as biological safety in general; procurement and calibration of testing equipment and tools; and other practical aspects of BSC maintenance.

In the closing ceremony, participants received training certificates and thanked the WHO Regional Office for Europe and all the course instructors for conducting this unique and very important training session in support of the national TB control programmes and laboratory biosafety in Georgia, Ukraine and Azerbaijan. Professor Zaza Avaliani, head of the Georgian NTP, congratulated the participants and faculty for successfully completing the course.

Annex 1
List of participants

Panel

Ms Svenja Schneider, Engineer, Germany

Mr Jörg Weber, Engineer, Germany

Dr Paul Jensen, Centers for Disease Control and Prevention (CDC), Atlanta, USA

Dr Grigory Volchenkov, Vladimir Regional TB Control Centre, Russian Federation

Dr Soudeh Ehsani, WHO Regional Office for Europe, Copenhagen, Denmark

Trainees

Shota Dvalishvili, Consultant engineer, National Centre for Tuberculosis and Lung Diseases, Tbilisi, Georgia

Samir Mirzakhanov, Engineer, Republican Anti-plague Station, Ministry of Health, Azerbaijan

Kamran Maksimov, Engineer, Penitentiary Sector, STI, Ministry of Justice, Azerbaijan

Tariel Kashia, Head of Control Division of Licensing Conditions Protection, LEPL State Regulation Agency for Medical Activities, Tbilisi, Georgia

Dr Roman Rodyna, Deputy General Director in Regional Development, Public Health Centre, Ministry of Health, Kyiv, Ukraine

Natalia Kampos-Rodriges, Laboratory specialist in TB diagnosis, Public Health Centre, Ministry of Health, Kyiv, Ukraine

Annex 2
Training agenda

WORLD HEALTH ORGANIZATION
REGIONAL OFFICE FOR EUROPE

WELTGESUNDHEITSORGANISATION
REGIONALBÜRO FÜR EUROPA

ORGANISATION MONDIALE DE LA SANTÉ
BUREAU RÉGIONAL DE L'EUROPE

ВСЕМИРНАЯ ОРГАНИЗАЦИЯ ЗДРАВООХРАНЕНИЯ
ЕВРОПЕЙСКОЕ РЕГИОНАЛЬНОЕ БЮРО

Subregional training on tuberculosis laboratory biosafety cabinet maintenance	
Tbilisi, Georgia	
29 August–6 September 2017	**Original: English**

Provisional programme: 29 August 2017

Theoretical – Day 1		
Tuesday 29 August 2017		
Time	**Topic**	**Lecturer**
09:00	Welcome and introduction	Soudeh Ehsani Zaza Avaliani
09:30–10:30	Biosafety I • biological agents • PPE • risk assessment	Svenja Schneider Grigory Volchenkov Paul Jensen
10:30–11:00	Coffee break	
11:00–12:15	Biosafety II • biological agents • PPE • risk assessment	Svenja Schneider Grigory Volchenkov Paul Jensen
12:15–12:45	Biological safety cabinets: standards and BSC classes	Svenja Schneider Paul Jensen
12:45–13:45	Lunch break	
13:45–15:15	Biological safety cabinets: standards and BSC classes	Svenja Schneider
15:15–15:45	BSC tests and test types: when, what, how often, by whom	Svenja Schneider

15:45–16:15	Coffee break	
16:15–17:00	BSC tests and test types: when, what, how often, by whom	Svenja Schneider
17:00–17:30	PPE	Grigory Volchenkov
	Panel discussion and take-home messages	Svenja Schneider Jörg Weber Paul Jensen Grigory Volchenkov Soudeh Ehsani

Provisional programme: 30 August 2017

Theoretical – Day 2		
Wednesday 30 August 2017		
Time	**Topic**	**Lecturer**
09:00–11:00	Protection test methods Test procedures (by manufacturer and by certifier) Impact of ventilation	Svenja Schneider Paul Jensen Grigory Volchenkov
11:00–11:30	Coffee break	
11:30–12:45	Decontamination	Svenja Schneider Paul Jensen
12:45–13:30	Lunch break	
13:30–14:15	Decontamination	Svenja Schneider Paul Jensen
14:15–15:00	Theory: airflow measurement	Svenja Schneider Paul Jensen
15:00–15:30	Coffee break	
15:30–17:00	Practical: airflow measurement	Paul Jensen Svenja Schneider
17:00–17:30	Panel discussion and take-home messages	Svenja Schneider Jörg Weber Paul Jensen Grigory Volchenkov Soudeh Ehsani

Provisional programme: 31 August 2017

Theoretical – Day 3		
Thursday 31 August 2017		
Time	**Topic**	**Lecturer**
09:00–11:00	Filter technology • HEPA filter • efficiency test • microbiological safety cabinets	Svenja Schneider Paul Jensen
11:00–11:30	Coffee break	
11:30–13:00	Theory of filter testing	Svenja Schneider Paul Jensen
13:00–13:45	Lunch break	
13:45–14:30	Types of test	Paul Jensen
14:30–15:15	Change, disposal and decontamination of filters	Svenja Schneider Paul Jensen
15:15–15:45	Coffee break	
15:45–16:45	Additional testing methods Types of testing	Svenja Schneider Paul Jensen
16:45–17:15	Written examination on theoretical knowledge	

Provisional programme: 1 September 2017

Practical – Day 1		
Friday 1 September 2017		
Time	**Topic**	**Lecturer**
09:00–10:00	Presentation and explanation of measurement instruments in general	Jörg Weber Paul Jensen
10:00–11:00	Demonstration of photometer and particle counter; measurement and differences Operation of smoke tests (lecture)	Jörg Weber Paul Jensen
	Smoke tests – practical exercise (hands-on BSCs)	Jörg Weber Grigory Volchenkov Paul Jensen
11:00–11:30	Coffee break	

11:30–12:30	Smoke tests – practical exercise (hands-on BSCs) (continued)	Jörg Weber Grigory Volchenkov Paul Jensen
12:30–13:00	Operation of velocity and airflow measuring devices and associated calculations	Jörg Weber Paul Jensen
13:00–14:00	Lunch break	
14:00–16:00	Velocity and airflow measuring – practical exercises (hands-on BSCs) *Indirect* • inflow velocity • inflow velocity (reduced sash height) • downflow velocity • outflow velocity *Direct* • DIM	Jörg Weber Grigory Volchenkov Paul Jensen
16:00–17:00	Parameters of airflow/velocity adjustment	Jörg Weber Paul Jensen
19:00–22:00	Joint dinner (departure from hotel at 19:00)	

Provisional programme: 4 September 2017

Practical – Day 2		
Monday 4 September 2017		
Time	**Topic**	**Lecturer**
09:00–09:45	UVGI	Grigory Volchenkov
09:45–10:15	Overview of the Tbilisi new NRL design and ventilation system	Paul Jensen
10:15–11:00	Discussion of downflow measurements carried out by participants on Practical Day 1	Paul Jensen Jörg Weber
11:00–11:30	Coffee break	
11:30–13:00	Inflow measurement – demonstration and measurement by participants using an anemometer Assessment of laboratory ventilation system	Paul Jensen Jörg Weber Grigory Volchenkov
13:00–14:00	Lunch break	

14:00–15:00	UV-C radiometer – demonstration and measurement	Grigory Volchenkov
15:00–15:30	Coffee break	
15:30–16:00	Discussion of down- and inflow measurements	Jörg Weber Paul Jensen
16:00–17:30	Downflow and exhaust filter testing using particle counter and photometer – demonstration and measurements by participants	Jörg Weber Grigory Volchenkov

Provisional programme: 5 September 2017

Practical – Day 3		
Tuesday 5 September 2017		
Time	**Topic**	**Lecturer**
09:00–10:30	Prefilter/first filter testing using a photometer and particle counter	Jörg Weber Grigory Volchenkov Paul Jensen
10:30–11:00	Reporting and recording • testing protocol • recording protocol	
11:00–11:30	Coffee break	
11:30–12:00	Electrical safety evaluation (grounding)	Jörg Weber Paul Jensen
12:00–13:00	Operation of BSC decontamination – formalin evaporator, operation and calculation	
13:00–14:00	Lunch break	
14:00–15:30	Decontamination practical exercise (hands-on BSCs)	Jörg Weber Grigory Volchenkov Paul Jensen
15:30–16:00	Coffee break	
16:00–17:00	Visit of operating TB NRL • biosafety measures • smoke test, downflow and inflow measured by participants and compared to factory acceptance test	Soudeh Ehsani Jörg Weber Paul Jensen

Provisional programme 6 September 2017

Practical – Day 4		
Wednesday 6 September 2017		
Time	**Topic**	**Lecturer**
09:00–11:00	Practical examination Recording of test results using a template test report for the following tests: • smoke test • alarm and control signals • inflow measurement • downflow measurement • filter integration/leakage test with particle counter	Jörg Weber Grigory Volchenkov
11:00–11:30	Coffee break	
11:30–14:00	Practical examination Recording of test results using a template test report for the following tests: • smoke test • alarm and control signals • inflow measurement • downflow measurement • filter integration/leakage test with particle counter	Jörg Weber Grigory Volchenkov
14:00–14:30	Lunch break	
14:30–15:30	Panel discussion	Jörg Weber Grigory Volchenkov Paul Jensen
15:30–16:00	Closing remarks	Zaza Avaliani Soudeh Ehsani Jörg Weber Grigory Volchenkov Paul Jensen