Technical Assistance for Management

REPORT
Detailed mission report and annexes

Mission n° 16SANIN107
Support the relocation of the National Reference Laboratory

Date of mission: from 11 to 18 May 2016

Albania

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Report by Véronique Vincent for TeAM/EF

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List of abbreviations

ACH	Air Changes per Hour
BCG	Bacille Calmette Guérin
BD	Becton Dickinson
BSC	Biosafety Cabinet
BSL	Biosafey Level
DST	Drug Susceptibility Testing
EQA	External Quality Assurance
FLD	First-Line anti TB drugs
FM	Fluorescence Microscopy
HEPA	High Efficiency Particulate Air
HIV	Human Immunodeficiency Virus
HVAC	Heating Ventilating and Air Conditioning
ICA	Immuno Chromatographic Assay
IQC	Internal Quality Control
IUATLD	International Union Against Tuberculosis and Lung Diseases
LJ	Löwenstein-Jensen
LPA	Line Probe Assay
MoH	Ministry of Health
MDR	Multi Drug resistant
MERV	Minimum Efficiency Reporting Value
MIRU-VNTR	Mycobacterial Interspersed Repetitive Unit – Variable Number Tandem Repeat
NRL	National Reference Laboratory
NTP	National TB Programme
PHC	Primary Health care Centres
PCR	Polymerase Chain Reaction
SLD	Second-Line anti TB drugs
SNRL	Supra National Reference Laboratory Network
TB	Tuberculosis
UHCT	University Hospital Centre of Tirana
UHSN	University Hospital "Shefqet Ndroqi"
ULPA	Ultra-Low Particulate Air
UV	Ultraviolet
VAV	Variable air volume
WHO	World Health Organization

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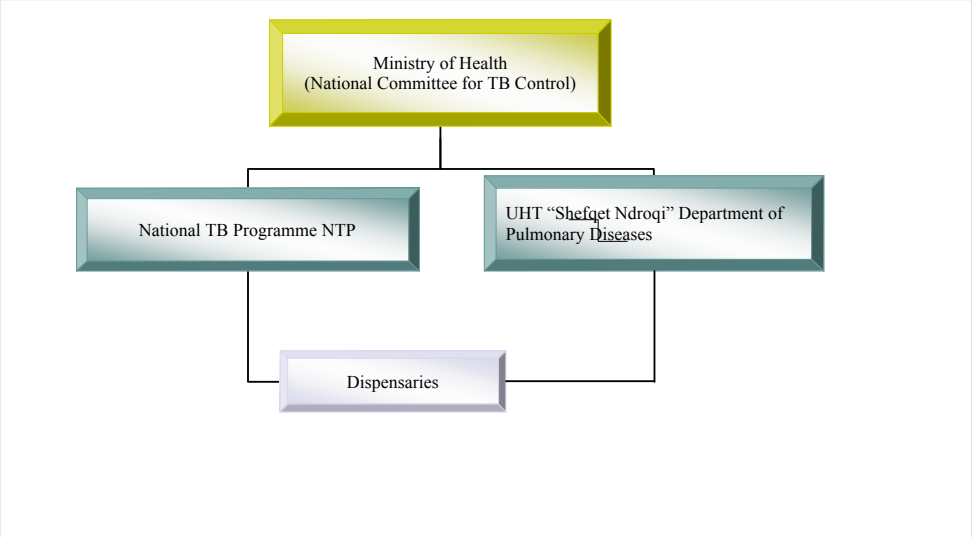
Background

Tuberculosis services include a broad range of services such as: diagnosis, treatment, and prophylaxis. With the exception of the BCG vaccination, all of the above mentioned services have been integrated under the pulmonology service since 1982. In the outpatient service, this service is delivered by the lung diseases dispensaries. The organization of tuberculosis control in Albania is organized vertically, i.e. it is a closed system within the pulmonology service, despite few efforts to integrate it within the primary health service.

The Ministry of Health is the main responsible institution for the control of tuberculosis, including the legislation and the budget for TB control. There is no dedicated tuberculosis budget. Only the budget for anti-tuberculosis drugs is separate, and this budget is allocated to the University Hospital "Shefqet Ndroqi" (UHSN), which is responsible for its management. As the hospital has many other priorities, TB dedicated funds are very limited, quite insufficient for the real needs. The Central Unit of the National TB Programme (NTP) and the national tuberculosis reference laboratory (NRL) are parts of the UHSN.

The following chart represents in broad lines the organization of tuberculosis in Albania:



TB laboratory service organization

As a rule family doctors should refer patients with respiratory complaints to a pulmonologist at a TB dispensary or at the hospital. Actually, patients often appear at dispensary personally without the recommendation of the family doctor. However, as incidence has declined, family doctors do not often think of TB and pulmonologists do not pay the necessary attention to TB. Therefore the orientation of suspects to TB diagnostic services is likely to be low.

The detection of TB cases is based on clinical, radiological and bacteriological examination by sputum smear microscopy. Among the 27 TB dispensaries, 13 have an operational laboratory with a microscope for sputum smear examination. The remaining 14 dispensaries have only one doctor with one or two nurses and no functional laboratory. Stock-out of reagents remains an urgent problem. The laboratory network consists of the 13 peripheral laboratories in dispensaries, 12

laboratories in the former lung disease hospitals or the regional general hospitals, the paediatric Mother Teresa hospital and the NRL at the UHSN in Tirana.

Objectives of the mission

The purpose of our mission is to provide recommendations for the rehabilitation of the NRL premises in line with international standards and for strengthening its activities and supervision role for the increase efficiency of the national TB laboratory network.

Findings

Activities of the NRL.

The NRL staff consists of the MD head of the laboratory and 5 technicians who rotate on a 6-month basis. Cleaning and waste elimination are carried out by 1 and ½ person. On average, 20 sputum samples per day are investigated for TB detection based on microscopy and culture processing of each sample. The NRL uses both liquid and solid media according to international standards. Solid Löwenstein-Jensen medium is prepared on site; liquid MGIT media and reagents are BD acquired. Positive strains are identified using the BD immunochromatographic (ICA) test. Positive strains of tubercle bacilli are tested against first-line drugs in liquid media to provide as early drug-susceptibility testing (DST) results as possible. The NRL is the only lab in the country that performs culture and DST. The private clinics can only perform direct smear microscopy (Ziehl-Neelsen) and send suspected samples for culture to the NRL.

Further differentiation and specific identification of tubercle bacilli is carried out with the Hain MTBC line probe assay (LPA). The NRL also implemented identification of mycobacterial species other than tubercle bacilli based on the Hain Genotype Mycobacterium LPA kit. Moreover, the NRL developed capacity and know-how for performing molecular genotyping of tubercle bacilli with spoligotyping and MIRU-VNTR typing methods. Both methods are international gold standards for determination of transmission groups, a useful epidemiological tool, and identification of phylogenetic families within the tubercle bacilli population.

Regarding DST quality control, the NRL has established collaboration with the WHO/IUATLD Supra National Reference Laboratory Network since 1999. The SNRL network organizes a yearly DST EQA test, consisting of a panel of 20 strains for blind testing and susceptibility against first-line drugs. Quality performance scores of the NRL were quite high with 100% compatibility in 2008 and 2010, and 90% for isoniazid and 100% for rifampin in 2013.

However, due to lack of reagents most of tests could not be performed these last years since 2013, especially affecting the number of DST performed. In other words, the NRL could not perform diagnostic activities and provide therapeutic indications for the proper management of TB cases. Actually, the NRL activities and its contribution to case detection and to case management are overlooked.

The laboratory network is weak as several laboratories still have poor infrastructure and face supply shortages.

The NRL premises and equipment
The NRL is located in the UHSN and occupies a large space, with dimensions quite adequate for its activities. However, rooms need to be refurbished and renovated, especially to match biosafety standards. At present there is no restricted access to the NRL. Laboratory rooms where the high-risk procedures are performed open out onto the corridor. The current layout does not allow the organization of a directional workflow from low- to moderate- to high-risk procedures or separation of "clean" and "hazardous" procedures. Biosafety cabinets, the key equipment for protection of technicians, are not well maintained. Two among the 4 BSCs cannot be used, one out of order, one without any filter. Most of the NRL equipment is out of order and prevent successful completion of techniques, especially culture procedure.

Role of the NRL

We emphasize the critical role of the NRL to achieve the goals of the new National TB Strategy 2015-2019. The goal of the strategy is to consolidate the achievements made so far in the fight against TB and to lead the country to the stage of TB elimination by the end of 2019.
A major component of the strategy is ensuring quality services for all TB patients nationwide for a fast and accurate bacteriological diagnosis. The NRL already provides bacteriologically confirmed diagnosis of TB by microscopy, culture, line-probe assay and drug susceptibility testing.
Thanks to the GF financial support, GenXpert will be implemented at the NRL for a rapid detection of TB and MDR TB. Moreover, the network of laboratories is being reorganized and strengthened to ensure culture for all patients across the country. A transportation system for sputum samples from the periphery throughout the country to the NRL will be organized.

Therefore, the budget, equipment, layout and workforce of the NRL should be well in line with challenges to increase detection of TB and drug resistant TB nationwide, and ensure quality and continuity of services.

Expected deliverables
- Recommended layout of the NRL in the new hospital premises and a preliminary list of the civil work required,
- Design of the mechanical ventilation of the NRL and its running costs,
- List of new laboratory equipment to be procured during the lifetime of the Global Fund grant,
- Revised guidelines for TB laboratory investigation and for airborne infection control.

RECOMMENDATIONS

To the MOH

- Allocate a specific budget to the NTP with a dedicated line for the NRL to cover needs for reagents and consumables for the NRL and for the TB laboratory network. It is of the utmost importance especially as the MOH is committed to financially support all consumables and reagents for microscopy and culture in Year 3 of the GF project and all needs of the NRL and lab network later on.
- Implement (by contracting specialized, qualified companies) and impose as mandatory a system to ensure maintenance of equipment in the laboratory.

To the UHSN, UHCT, NTP and NRL

- Improve the access of TB suspects and TB contacts to TB diagnosis, as it is likely that not all presumptive TB suspects are timely assessed for TB.

- Involve family doctors in the referral of TB suspects.
- Appoint a TB coordinator in UHCT for paediatric patients
- Train medical staff on TB and TB diagnosis
- Develop flyers on TB symptoms for distribution to family doctors and PHC to increase TB awareness and inform patients that a productive cough for more than 2 weeks may be a TB symptom.
- Include questions about cough occurrence in the systematic patient questionnaire at PHC medical visit for increasing orientation of suspects to TB centres.
- Provide diagnostic means in dispensaries and other TB related facilities (chest X-ray, sputum examination)

- Strengthen the laboratory network

- Provide adequate stocks of sputum containers and transportation boxes to all centres. A refrigerator, a well functioning microscope and microscopy reagents should be available in dispensaries with laboratories.
- Organize training sessions covering the logistics of the transportation system. At least one person from all centres participating in the referral system, i.e. the 28 dispensaries, 12 regional hospitals including the 3 lung hospitals, and Mother Teresa hospital should participate.
- Detail the specifications of the contract with the private company in charge of the transportation system to gain benefit of a regular and timely transportation of specimens. Specifications should be strict on cold transport conditions and timely collection of specimens. Confirm if the average cost of 10 USD per sample as forecasted is realistic according to distances between the NRL and the busiest dispensaries.

- Increase the NRL diagnostic capacity

- Reorganize the laboratory layout according to international standards, especially regarding biosafety protection measures, adopting robust and sustainable options
- All equipment and consumables should meet specifications described in the WHO document: Guidance for countries on the specifications for managing TB laboratory equipments and supplies, available at apps.who.int/iris/bitstream/10665/44798/1/9789241503068_eng.pdf
- Ensure maintenance and annual certification of the Biosafety Cabinets
- Implement rapid methods for TB diagnosis and drug resistance identification (GenXpert)
- Ensure continuity of services with continuous availability of good quality supplies

- Monitor the performance of the laboratory network using operational indicators of quality assurance
- Staff the NRL to adjust the workforce to the estimates of the future workload
- Train staff of the NRL and peripheral laboratories to new diagnostic techniques and TB infection control
- Sustain involvement of the NRL in operational research
- Implement a regular medical supervision for all TB laboratory workers to monitor for occupationally TB infection. All cases of disease identified as resulting from occupational exposure shall be notified.

To the Global Fund

Associate the head of the NRL to adjustments/revision of the budget to be sure that no major issue is omitted or undervalued at the risk to affect and weaken the quality of TB lab services

To CCM

Check that needs of the NRL and TB laboratory network are well addressed during the roll-out phase of the GF grant

To WHO

Organize advocacy meetings and campaign for TB awareness

ACTIVITIES CONDUCTED

- Visit of the NRL
- Visit of the new laboratory premise
- Meetings with the NTP manager
- Visit of the NRL (present and anticipated location)
- Visit of the dispensary in Durres
- Visit of a private radiological clinics in Durres
- Visit of the hospital laboratory in Durres
- Desk review of documents
- Review of background and sections of the draft report
- Team meetings
- Briefing/debriefing with the Director of the UHSN
- Briefing/debriefing with the Attachée de Coopération, French Embassy
- Debriefing with the CCM vice-chair

LESSONS LEARNED AND RECOMMENDATIONS

- Collaboration with the national consultant, the head of the NRL and the NTP manager was very active and efficient
- Timing of the mission allowed confirmation of the MOH agreement for the implementation of the GF program with scheduled timeframe for the beginning of activities by September 2016
- During the mission (6 days after start), the allocation of the new premise at the UHSN for the laboratory was not confirmed and decision was taken by the Director Pr Perlat Kapisyzi to have renovation in the current premises.
- Support from Isabelle Thomas-Delic was very appreciated and contributed to enhanced commitment of the Director of the Hospital to the project
- Our recommendations are in line with WHO biosafety recommendations and emphasize robust and sustainable solutions

AGENDA

Wednesday 11 May

9 00-10 00 Meeting at the French Embassy
10 30-11 00 Meeting with the Head of the WHO Regional Office
11 30- 13 00 Visit of the actual NRL
13 00-14 00 Lunch
14 00- 16 00 Visit of the new premise

Thursday 12 May

10 00- 100 Meeting at UNAIDS
11 00-12 30 Meeting with the NTP manager
12 30-13 30 Lunch
13 30-16 00 Discussion at the NRL on equipment and diagnostic algorithms

Friday 13 May

09 00-10 00 Travel to Durres
10 00 -12 00 Visit of the TB Dispensary
12 00-13 00 Visit of the private radiological clinics
13 00-14 00 Meeting with the Director of the Hospital
14 00 -15 00 Visit of the laboratory of the Hospital
15 00 -17 00 Lunch and travel back to Tirana

Saturday 14 May

Desk review of the documents
Development of the report: layout of the NRL in the new premise, revised list of equipment needed

Monday 16 May

09 30 13 00 Review of list of equipment and discussion on the referral system within the laboratory network
13 00 14 00 Meeting with the Director of the UHSN for presentation of the proposed layout: decision taken not to move the NRL and to renovate the old premise
14 00 16 00 Revised layout of the NRL in the current old premise

Tuesday 17 May

09 30 13 00 Review of the layout in the renovated NRL with placement of equipment in newly designed laboratory rooms
13 00 15 00 Description of modalities for the referral system within the laboratory network

Wednesday 18 May

09 00 10 00 Debriefing with Isabelle Thomas-Delic at the UHSN
10 00 13 00 Discussion on documentation and new WHO policies
13 00 14 00 Debriefing with the CCM vice-chair

LIST OF PERSONS MET

Dr. Silva Tafaj – Head of the NRL for TB
Isabelle Thomas-Delic – Attachée de cooperation, Ambassade de France en Albanie
Dr. Ledia Lazeri – WHO Representative, Albania
Bujana Hoti – Coordinator HIV Dept, UNAIDS
Dr. Donika Mema – NTP Manager
Dr. Anila Aliko – TB Infection Control consultant
Dr Zyber Pupla – pulmonologist, Head of the Durres dispensary

Dr Edlira Ndreu – pulmonologist, TB ward Durres Hospital
Dr Fatmira Luli – Head of the radiological clinics in Durres
Mr Alban Ramohitaj – Director of the Durres Hospital
Dr Endri Shehu – Vice-Director of the Durres Hospital
Dr Dolores Koka – Head of the laboratory of the Durres Hospital
Prof. Perlat Kapisyzi – Director of the UHSN, Head of Pneumology 1 Department
Mrs Olimbi Hoxhaj – CCM Vice-chair

DELIVERABLE 1. Recommended layout of the NRL in its actual location, and preliminary list of the civil work required

Background

Following discussion with the Director of the Hospital, we understood there is another plan, well established, regarding the organization the new premise, where TB laboratory was supposed to move. In this plan, space is allocated to bronchoscopy and gastro-endoscopy.

The Director of the Hospital asked us to think on another plan of rehabilitation of the TB laboratory, in the location where it's functioning now.

After analyzing the premise, we considered as acceptable to rehabilitate the TB laboratory in the location where it is currently operating, after several changes will be done.

Description of the laboratory proposed layout after refurbishment in the location where it's functioning at present

- The laboratory will occupy the same space as before, in neighbourhood of the haematology and biochemistry laboratories, with access through a common hallway.
- Reception of specimens will be carried out in this area as already in place.
- The entrance in the laboratory will be restricted by a door allowing limited access (electronically or by key) only for authorized staff. Door will be located in the same place it is now or may be moved 50 cm toward exit, increasing the surface of the lab reception area.
- A second door will be placed at 2,5m from the first, delimiting between them a space of 3m/2,5m (***Room#0***) that will be functioning as a dressing room in the laboratory, where staff will undress/dress and wear protective equipment.

Room#0 will be provided with two other communications:

-one entrance on the right to ***Room#1,*** which will be separated by a glass wall to provide an office for the staff for paper and computer work on the window side and create a space for molecular procedures (post PCR processing).

-one entrance in front of the main door, leading into laboratory's corridor.

Patient's (or other unauthorized person's) access into the laboratory will not be allowed. Verbal communication system (interphone) will be installed outside the laboratory for communicating with staff.

The laboratory will be divided in two functional areas: one „clean" area, on the right side of the central corridor and one "potentially contaminated" area on the left side; "Potentially contaminated area" or containment area will have only one access door that will lead to ***Room#2*** which is currently an antechamber to the walk-in incubator room ***(Room#3);***

Room#2, Room#3 and Room#4 will be provided with large communication with each other, as much as the building structure allows that. The wall between ***Room#4*** and ***Room#5*** will be moved to allocate more space for the moderate–risk area where all specimens will be processed. Space formed by the three rooms will be functioning for:

Low-risk procedures:
-opening the containers with initial processing of the specimens (direct sputum smear microscopy); the procedures will be done on a benchmark in a simple hood or in a class I biosafety cabinet (that is already available. Although not currently used due to biosafety issues, it may be used for this low-risk procedure without HEPA filter)
-preparation of the specimen for use in an automated nucleic acid amplification test cartridge (GenXpert MDT/Rif assay)

Moderate risk procedures
-liquefaction/concentration of the specimens (centrifugation with safety buckets); during the centrifugation process, buckets must be hermetically sealed with aerosol-free covers and after centrifugation buckets have to be opened in the BSC as aerosols may be produced.
-processing and concentration of the specimens for inoculation on primary culture media
-direct DST (for example line probe assay on processed sputum)
The procedures with moderate risk of generating infectious aerosols will be performed in a biosafety cabinet (class II), which provides the primary containment of infectious aerosols generated by these procedures.

Room #5 is the **containment room** designated for *high-risk procedures* for generating infectious aerosols:
- culture manipulation for identification, phenotypic DST, or DNA extraction from cultured isolates.
The procedures with high risk of generating infectious aerosols will be performed only in biosafety cabinet (class II), which provides the primary containment of infectious aerosols generated by these procedures.
In Room#5 will be located the MGIT machine and incubators of cultures.
The door allowing at present communication of the room#5 with the hallway will be sealed, replaced by a glass wall.
Room#5 will reduce its dimensions by wall movement / construction of a glass wall.

Room #6: sterilization room
A communication (crossing counter) will allow the transfer of the recipients that need to be autoclaved from Room#5 to Room#6.
Room#6 will host 2 autoclaves, one to sterilize solutions or glassware and one to decontaminate infectious materials.

Room#7 is designated for glass recipients washing and drying

Room #8 will be dedicated to the staff (rest area)

Room#9 will be an antechamber between two clean areas –Room#10 (pre PCR processing) and Room#11 (media preparation)

Room#10 will be dedicated for pre-PCR steps that mean DNA-free reagent preparation. It will host a laminar flow cabinet (clean bench) and a freezer.

Room#11 will be separated by a glass wall to create an office for staff for paper work on the window side and to leave enough space on the corridor side for media preparation

Room #12 will be designated as office for the head of the laboratory

Room #13 will be designated for General Microbiology (nonspecific flora processing) and for the intermediate step of TB PCR (adding TB DNAs to PCR mix). A glass window will delimitate a room corner for a bench dedicated to this molecular work.

Room#14 will be further re-organized to be a storeroom
Microscopic examination will be performed in a space organized in the main corridor or in Room#1.

Preliminary list of civil works required

1. Analyze and agreement of the management of the UHSN of the proposed layout of the laboratory
2. Evacuation of old, out-of-order equipment
3. Elimination of hazardous chemicals, still stored in the laboratory although of no use
4. Commissioning of the work to specialized Company
5. Changes to be done to the actual building:
    a. Installing of a door allowing limited access only for authorized staff at the entrance to the laboratory.
    b. Installing a second door placed at 2,5m in front of the main entrance door
    c. Creation of a separation (glass wall) in room#1 to create an office space on the window side and create a space for microscopy on the corridor side
    d. Creation of a separation (glass wall) in room#11 to create an office space on the window side and create a space for media preparation on the corridor side
    e. Creation of a separation (glass wall) to delimitate a room corner for a bench dedicated to this molecular step.
    f. Demolition of the thermostat room (hot room); communication to be created between Room#2 (actual thermostat antechamber) and Room#4 for creating a medium risk area.
    g. Moving the wall between room#4 and room#5 to allocate more space to room 4
    h. Sealing of the door that is at present allowing the access from the corridor to culture processing area (room#4)
    i. Sealing of the door that is at present allowing the access from the corridor to high risk procedure room (room#5)
    j. Constructing of a communication (crossing counter) between room#5 (containment room) and room #6 (autoclave room); this will consist of two glass windows that will delimitate a space in between them. One window will be operable to room#5, to allow the transfer of the recipients that need to be autoclaved in the intermediate space and then, by opening the second window to be taken in the next room, room #6 (sterilization room). The two windows of the crossing counter will be never opened simultaneously, as the space in between them will act as a buffer area in between the two spaces.

k. Installation of mechanical ventilation devices (fans, pipes, diffusers, grills) if mechanical ventilation will be chosen as a solution
l. Revision of the electrical system (placement of power supplies, installation of a protection system - UPS)
m. Revision of the sanitary installation (water supply and drainage) according to the needs of the equipment
n. Creation of a separation (glass wall) in room#11 to create an office space on the window side and create a space for media preparation

6. Installing of the equipment (only by authorized and competent personnel).

Figure 1. ***Proposed new layout of the renovated NRL***

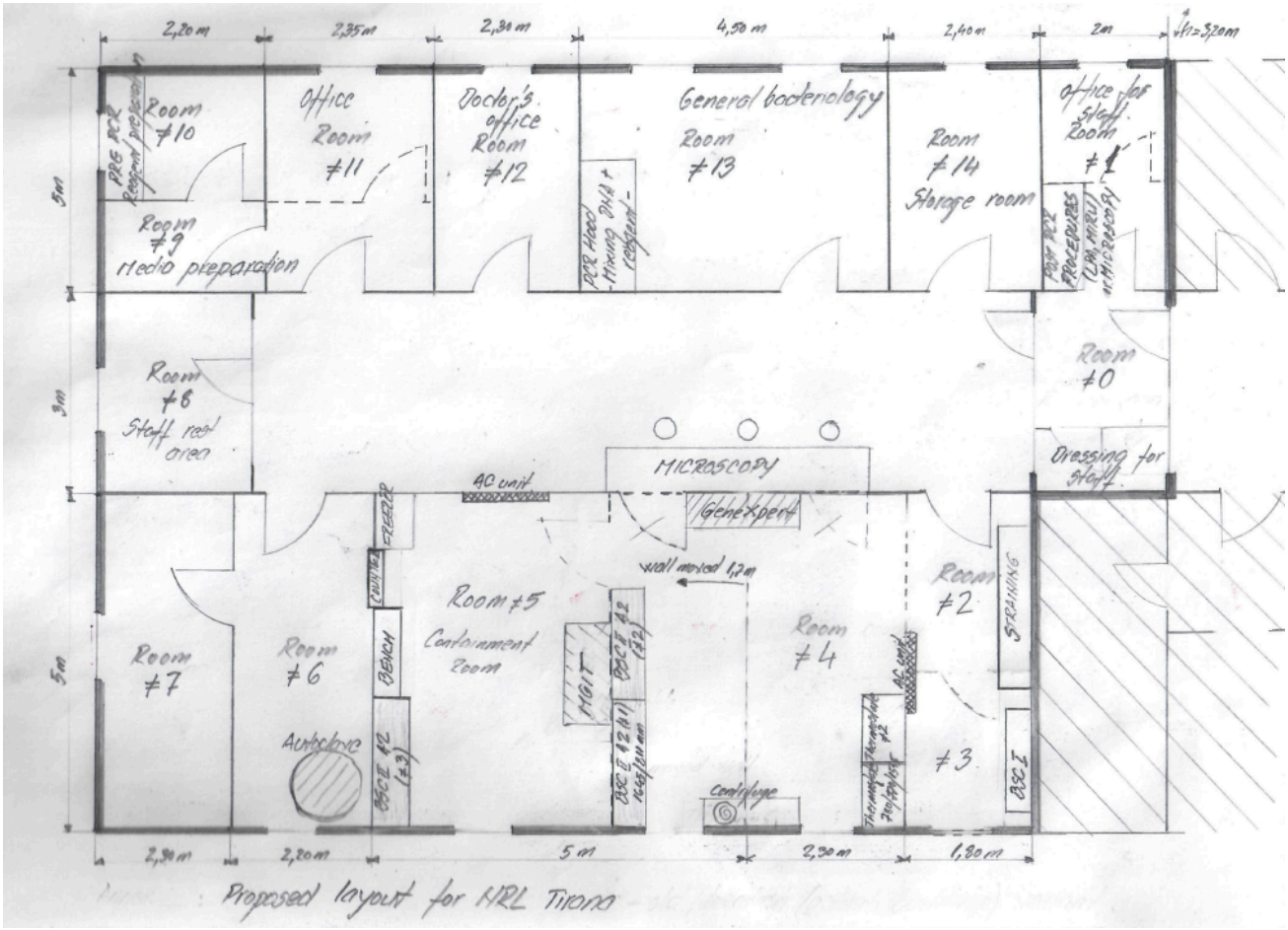


Figure 2 ***Current layout of the NRL***

DELIVERABLE 2. Design of the mechanical ventilation of the NRL Tirana and its running costs

M. tuberculosis is classified as a Risk Group 3 pathogen, requiring special environmental conditions in the laboratory: the use of appropriate negative pressure systems, biological safety cabinets (BSC), aerosol-free centrifuges, and a whole range of related laboratory equipment and supplies. WHO described the design of laboratories corresponding to the manipulation of pathogen classified according to risk groups in the "Laboratory biosafety manual" published in 2004. However, in 2012, WHO published a "Tuberculosis laboratory biosafety manual" to specify **minimum requirements** needed to mitigate risks of infection in laboratories dealing with TB bacilli, **based on assessments of the risks** associated with the different technical procedures. The most hazardous techniques as per the manipulation of TB cultures have to be carried out in TB-containment laboratories which may or may not meet all the requirements of a Biosafety Level (BSL) III laboratory as described in the 2004 WHO manual. The recommendations below are in line with the WHO guidelines described in the TB laboratory biosafety manual, especially for the TB-containment laboratory.

According to the procedures performed at the NRL in Tirana, the risk of airborne transmission is moderate to high; limiting the area where risk manoeuvres are taking place in only two rooms is the first step to decrease the risk.

All procedures involving processing of potential infectious samples and manipulation of live cultures will be conducted in a BSC. The BSC is the primary form of containment while specimens are processed for culture inoculation, performing DST or molecular biology procedures. Proper use of the BSC is critical to allow work to be conducted safely. In addition to the BSC, the secondary barrier of containment is provided by the laboratory itself; this is achieved by maintaining unidirectional airflow into the laboratory from "clean area" to "potentially contaminated area" and by ensuring there is good air mixing (6-12 ACH). This may be possible using the negative pressure produced by the BSCs, if they are ducted to the outside.

Alternatively, a dedicated mechanical ventilation system may be used. WHO does not impose as mandatory the installation of mechanical ventilation systems in BSL III laboratory. Mechanical ventilation systems are expensive, regarding construction, running cost and maintenance. They request engineering knowledge and complex technical devices in order to maintain constant climate (temperature, humidity), differential pressure and directional airflow in the laboratory, to insure comfort (acceptable noise, draft) and biosafety conditions for the laboratory workers as well as for all persons (adjacent staff, patients) circulating in the building.

Taking into consideration high construction, electricity consumption and maintenance costs, poor maintenance of the infrastructure that we noted, but also the low number of MDR TB strains expected to be processed at the NRL, we do not recommend the implementation of a complex mechanical ventilation system and we consider as realistic, robust, sustainable and in line with biosafety requirements the use of natural ventilation in association with directional airflow provided by the BSCs ducted to the outside.

Variant #1 (recommended): Natural ventilation and containment provided by the BSCs

This is the easiest and cheapest, being first choice for setting the ventilation system in the NRL at UHSN in order to assure proper infection control.
At this moment, in the actual premise of the NRL are installed 4 BSCs, 3 class II type B2 and one class I out of order. The class II type B2 BSCs have an average age of 10 years and were not properly maintained, as there is no service in place; the inferior filter of one BSC (that is an ULPA filter) was changed with similar filter taken from the other BSC that was less used. The engineer of the hospital who has no special training performed the operation, which is against all biosafety measures. That's why we have concerns related to the good shape of the BSCs and we recommend identification of a company to assess their functionality and costs for maintenance.

We recommend the acquisition of 3 new class II type A2 BSCs, the WHO preferred type for the following reasons:
-The advantages of the class II type A2 BSC are lower operating and maintenance costs, as they are re-circulating 70% of the air to the work area as the downward airflow. Air, heat and energy loss are not high with this model of BSC.
-In the class II type B2 BSC all inward and downward airflow is exhausted after ULPA (the highest filtration grade, beyond WHO recommendations of HEPA filters) filtration to the external environment without recirculation within the BSC. Type B2 BSCs are suitable for work with toxic chemicals employed as an adjunct to microbiological processes under all circumstances since no re-circulation occurs (that is not the case in the TB laboratory). ULPA filters are much more expensive than HEPA filters. Moreover, type B2 BSCs are, in practice, difficult to install, balance and maintain. Moreover, the 2 type B2 BSCs already in place at the NRL should be revised, filters should be procured for at least one of them (filters have been moved from one BSC to the other) and both BSC should be certified after re-installation in the renovated laboratory. Therefore, in addition to the high running cost and maintenance, a budget should be identified for the re-installation of the BSCs. Because certification, operation and maintenance of these BSCs are more difficult, WHO does not recommended their use for any new TB laboratory facilities.

Use of the BSCs combined with natural ventilation should provide enough safe environments if they are proper installed and maintained.
Two BSCs will be installed in the medium risk area (room#4) and one BSC in the high-risk area (containment room#5).

How much ventilation will create the BSCs?
According to the type of the BSC that will be used and the volume of the space where they will be installed, there are some considerations to be taken into account:

- If class II type B2 BSCs already existent will be used, they have to be evaluated and periodically maintained with replacement of costly ULPA filters; one type B2 BSC provides 2000 m^3/hour air exhaust; for a 60 m^3 volume (as room#5 or space composed by rooms #2, #3 and#4), the type B2 BSC will provide ≈ 30 ACH, well beyond the minimum 6-12 ACH required for proper air dilution and directional airflow.

- If class II type A2 BSCs (that we recommend) will be used, their regular maintenance will be less costly; one type A2 BSC provides 500 m^3/hour air exhaust; for the same volume (60 m^3), it may provide ≈ 8 ACH, enough for proper air dilution and directional airflow. If 2 BSCs are used in the same space, they may provide double ventilation (≈ 16 ACH)

Supply Air Requirements for BSCs

Supply air requirements for BSCs are often overlooked. However, supply air requirements are as critical as exhaust air requirements to ensure proper BSC function. Whatever volume of air is exhausted from the BSC, air must be supplied to the room in order to avoid "starving" the cabinet of air. The supply air available to the BSC should be verified as well as the supply air to maintain desired room pressurization and air exchange rates. A BSC cannot be certified if a lack of supply air causes a low or inconsistent inward or downward airflow velocity.

In order to avoid depressurization of the rooms and wrong air direction from room #5 to room #4, air must be supplied from the outside into the rooms where BSCs are working; this may be done actively (with mechanical ventilation) or passively, providing windows with grills which permit air to come in the room from the outside. Grills (20/50 cm) will be installed in the lower panels of the laboratory doors; they will be unidirectional, permitting air to pass only in one direction, from lower to high-risk areas. This will allow the air to move from other rooms of the laboratory (that are not risk areas) to the BSC, creating directional airflow. As communication with the laboratory will be minimal, grills will consist of:

- one unidirectional grill placed on the door between hallway and room #2, allowing air to come from the hallway to the risk area
- second unidirectional grill placed on the door between room #4 and room #5; this will allow air from medium risk area (room #4) to go to high risk area (room #5) but not to come back if the pressure becomes "more negative" in room #4; this may happen especially if 2 BSCs are used in room #4;

Doors between the room 4 and 5 will be closed when BSCs are operating.

It has to be noted that with unidirectional grills, air only enters the room when BSC is working, but not while the BSC is at rest, avoiding heat loss. Grills will be equipped with pre-filters to minimize dust circulation into the room

Air exhausted from the BSCs must be conducted by pipes to at least 9m from any air supply (including windows), that means on the roof of the building.

- class II type B2 BSC must be hard ducted. Therefore the building's exhaust system must precisely match the airflow requirements specified by the manufacturer for both volume and static pressure.
- class II type A2 BSC must be ducted to outside by canopy or Flex Air type connection. The major advantage of a thimble connected BSC is that no adjustments need to be made to the cabinet and the direction of air flowing from the laboratory to the outside will be maintained.

Placement of type A2 BSCs is indicated in Figure 2 below. It has to be noted that in room #4, the 2 BSCs are placed on the wall directed to room #5:

- to avoid obstructing the windows (if they were placed on the exterior wall)
- to create optimum airflow from the door to the BSC, avoiding turbulences
- there is enough space for the 2 BSCs of 1,645m each on that wall (wall length is 5m, the door is 1m)

AC units must be placed where they do not interfere with airflow created by the BSCs. In room #2, #3 and #4 we can place one unit in room #2 or #3, as air will be well mixed, ensuring uniform cooling. In room #5, best placement is on the wall from the hall, least interfering with airflow produced by the BSC.

Variant 2: Mechanical ventilation system (HVAC – heating, ventilating and air conditioning) in addition to the BSCs (not recommended because of high cost of construction and maintenance)

Setting up complex mechanical ventilation system may create safer environment in the laboratory, but involves high responsibilities, is expensive and difficult to be maintained. The HVAC system functions not only for the control of infection, dilution and expelling of contaminants, but also to maintain minimum requirements of comfort and ventilation and to remove noxious odours. A ventilation system without air conditioning is not acceptable, as it cause discomfort to the people and are not used.

Basic principle of the mechanical ventilation in the laboratory
Mechanical ventilation system is designed to create directional airflow from clean areas, provided with "positive pressure" to potential infectious area, where "negative" pressure is created. Each room is provided with air exhausts and inlets. To create positive airflow, more air is introduced than removed from the room and to create "negative" pressure, more air is removed than introduced.
- The procedure rooms will be provided with negative pressure (being provided with dampers, to balance the exhaust created by the BSC).
- "Clean rooms" and "neutral rooms" may be provided with positive pressure or may have natural ventilation.

The exhaust air from these spaces shall not be re-circulate to any other area of the building, but shall be transported through dedicated exhaust ductwork to be directly discharged to the outside of the building.

Inflow and exhaust grills and diffusers will be placed in the rooms on opposite walls, to create directional airflow. It is recommended that inflow diffusers to be placed in the bottom of the wall and exhaust diffusers to be placed on the top of the wall (or on the ceiling), on the opposite wall.

All the windows in the ventilated area will be sealed, in order to avoid pressure imbalances.

Doors inside the laboratory (not at entrance or exit) will be provided with grills that will allow directional flow.

Doors will be provided with mechanical systems to keep them close (automatic

closing systems)

Mechanical ventilation system (HVAC) only in risk areas, in addition to the biosafety cabinets

One compromised solution should be to organize the HVAC system only in the risk areas, providing negative pressure in rooms #2, #3, #4 and #5 (containment laboratory), as indicated in Figure 1 below.
Principles are similar with those previously presented, but the area that should be ventilated is limited only to the procedures rooms; the cost of the system will decrease, as part of the components will be in smaller number; however ventilation controllers, heating and cooling system, air filtration, organizing of the exhaust and inflow are still compulsory and similar with the entire system ventilation.

Design of the ventilation system for the laboratory

HVAC system may be designated to provide 12 ACH and directional airflow (negative pressure in the potential contaminated area)

Mechanical ventilation system (HVAC) in addition to the BSCs should provide additional safety and comfort for the TB lab workers, if properly designed and installed.
-If actual Class II type B2 BSCs will be used, they provide enough high amount of air exhaust, making unnecessary additional exhaust; the role of HVAC should be to provide enough air during the time they are functioning.
-If Class II Type A2 BSCs will be procured and used, then HVAC system will be functioning additionally to them.

Mechanical ventilation system will be designed to provide at least 12 ACH and negative pressure in the medium and high-risk areas in relation with the other building. Negative pressure will conduct to directional airflow only from the laboratory to the potential contaminated area; potential infectious air will “be trapped” into the containment area.

Position of diffusers and grills should not interfere with BSCs activity.

Automatic control system (interlock, dampers) must coordinate HVAC capacity to the activity of the BSC: when the BSC is turned on, ventilation will automatically decrease its activity with equivalent air volume provided by the BSC.

Heating, cooling, possibly humidification of the inflow air is needed to provide comfort to occupants and good functionality of the devices in the laboratory.

Suggested design of the ventilation system in the laboratory, as airflow rates calculation is provided in Figure 1 below.

Estimated cost of the mechanical ventilation system:
Air fixtures (diffusers, grills): 1.500Euro
Pipes (ducts ≈ 60m): 3.000 Euro
Outdoors air intakes, air exhausters (including air filtering system): 2000 Euro
Controls: balancing provisions - dampers, Variable air volume, variable volume controls, Handling Unit Controls: 4.500 Euro
Air heating system, air-cooling system: 9.000 Euro
Engines and ventilators (fans): 5.000 Euro
Design and installation (including maintenance for next 5 years): 5.000 Euro
Total: ≈30.000 Euro at installation
In addition, the running cost for high electricity consumption has to be budgeted.

Technical advises for the constructor of the HVAC system

Exhaust Airflow and Static Pressure of BSCs
BSCs are constant volumetric airflow devices, and the fan energy to exhaust the cabinet must be provided by the building exhaust system. They will not function properly and cannot be certified if the exhaust flow or static pressure is not sufficient (or potentially if either is too high). Static pressure is the resistance produced by the friction of the air with the ducts. If static pressure is too high (for example ducts with low diameter or inappropriate configuration), it will produce equipment malfunction. In addition to the exhaust airflow, the static pressure requirements for hard-connected type B BSCs are relatively high. This is due to the resistance added to pull air through the BSC and its exhaust HEPA filters. For canopy-connected type A2 BSCs, the cabinet blower overcomes the resistance of the exhaust filter; however, sufficient exhaust flow is still important.
Proper HVAC design and operation is crucial to the proper operation of a BSC.
Air conditioning and heating systems should be verified to maintain the desired environmental conditions when BSCs are in operation, as well as when cabinets are turned off.
More crucial to the safety of laboratory workers and the integrity of aseptic work processes is the possible impact of air currents in the room on the performance of the BSC itself. The location of room air supplies and returns is critical as cross-drafts may negatively affect the performance of BSCs.

HVAC and BSC controls
Low exhaust flow can lead to a loss of containment at the front access opening of the BSC and pose a risk to workers. Exhausted BSCs should have airflow monitors that alarm when the exhaust flow is too low. The alarm function should be verified periodically. It is often desirable, or even required, to control the BSC and HVAC systems in concert. Interlocks in the control allow the room to maintain the required pressurization, air changes, and exhaust flow when the cabinet is in operation, as well as when the cabinet is turned off.

Volume and Negative static pressure Requirements
Because the remote blower must pull the exhaust air through part of the BSC ductwork and its exhaust HEPA filter, the initial negative static pressure required by the cabinet at its connection to the system may be 1.5 inches H_2O (38 mm H_2O) or higher. As the exhaust HEPA filter loads, the negative static pressure will need to increase in order to maintain constant airflows. Depending on the design of the cabinet, and its filter, the exhaust system may need to have a reserve of an additional 2 inches H_2O (50 mm H_2O) to obtain the maximum exhaust HEPA filter life.

Exhaust Volume Fluctuation and its Impact

The supply blower(s) in a type B BSC delivers a constant volume of filtered air downward through the work area during normal operation. Fluctuations in the exhaust volume will directly impact the inflow volume and thus the average inflow velocity.
This impact can be calculated by dividing the change in exhaust volume by the area of the work access opening (the height of the sash multiplied by the width of the opening). In normal operation, the exhaust volume should not fluctuate more than +/- 5% in order to maintain consistent cabinet containment. If the exhaust volume were to decrease 10%, the calculation yields a drop in the BSCs face velocity of 20 FPM (0,1016 m/s) with a potential loss of containment.
Note: This demanding requirement for consistent exhaust volume must be carefully considered when selecting a Type B2 BSC. Limiting exhaust variation to 5% or less exceeds the stated tolerance of most constant volume airflow controls.

Variable Exhaust Controls. HVAC controls for laboratories or other spaces having equipment requiring variable or intermittent exhaust requirements, shall be provided which maintain the required room relative pressurization and room conditions for all modes of operation of the equipment (i.e., on or off).

Chilled water and steam/hot water generators may be dedicated to the HVAC system, located on the top of the building.

Air Filtration. Minimum Efficiency Reporting Value (MERV) 8 "roughing" filters shall be provided upstream of all coils, velocity sensing devices, or other devices requiring protection from dust accumulation. “Roll filters”, cleanable media, or other filtration systems requiring more intensive maintenance should be avoided. Designers shall carefully consider the location of filters relative to humidifiers to minimize the possibility of wetting the filter media.

Humidity controls shall be provided as necessary to meet the requirements given for individual spaces. Air handling system humidification shall be achieved utilizing direct steam injection. Designers are responsible to designate the location of steam injectors relative to ductwork and air handling unit components, and so design them as to minimize concerns with moisture collection in/on the downstream elements. Provide a minimum of 3 m of straight ductwork, with no takeoffs, reducers, duct lining, or other components, immediately downstream of the injection location.

Variable air volume (VAV) Air Handling Unit Controls. All VAV systems shall be provided with supply and return fans, with economizer operation where required and where economically life cycle cost effective. Fan speeds shall be modulated by means of variable speed drivers. Supply fans shall modulate based upon maintaining a fixed static pressure at a location remotely located in the ductwork sufficient to assure operation of all VAV terminal devices. Supply, return, and outside airflow rates shall be measured by the direct digital control system, and the return fan shall modulate to maintain a fixed differential airflow below that of the supply fan. A high supply duct static sensor and shutdown capability shall be provided.

Outdoor Air Intakes. Outdoor air intakes shall be located as far as practical, but not less than 9000 mm (30 ft), from exhaust outlets of ventilation systems, cooling towers, combustion equipment stacks, medical/surgical vacuum systems exhaust, plumbing vent stacks, emergency generator exhaust, or from areas which may collect vehicular exhaust and other noxious fumes. Further consideration to be addressed to the wind direction and velocity, building geometry, and characteristics of the contaminant stream.

Noise Control Noise Criteria for individual rooms and spaces in the facility to be in accord with national legislation. Usually, noise produced by the ventilation system inside the rooms

has to be lower than 40-50 db.

Air Fixtures Air distribution supply, return, and exhaust fixtures (diffusers, grills, etc.,) shall be sized to provide air inlet/outlet velocities consistent with requested airflow. Designers must be aware that diffuser manufacturer's published noise characteristics are based upon idealized inlet conditions: crinkled flex duct, abrupt branch duct connections, elbows located immediately at the diffuser collar, and similar poor connections may result in unacceptable noise levels. Spin-in or other 90 degree duct drop connections to diffusers shall be equipped with equalizing grids as necessary to assure uniform air distribution at the diffuser inlets. Access panels shall be provided as necessary for access to fire dampers, smoke dampers, and control equipment, and to facilitate periodic cleaning or disinfecting of ductwork.

*Balancing Provisions***.** Duct branches serving each individual space shall be provided with a manual-balancing damper, accessible above the ceiling, located as remote from the space supply or return fixture (diffuser, register, etc.) as practicable. The balancing damper provided as part of air diffusers is not to be used for system balancing.

*Exhaust Systems***.** All exhaust discharge outlets shall be located above the building roof and located to prevent short-circuiting to air intakes or other building openings. Exhaust fans shall be located at the end of the exhaust duct run (exhaust ducts to be under negative pressure).

Additional air disinfection using UV light in the TB laboratory (recommended in addition to Variant#1 or #2)

Instead or in addition to the ventilation system, we suggest use of upper room UV fixtures, 30W, with horizontal flow of radiation (louvered) in the high-risk areas and in the hallway; one UV lamp is enough to cover 18-20 m^2 of the surface of the wall. We recommend the installation of UV lamps in the risk area for obvious reasons and also in the hallway (no risk area) to create a kind of air filter, protecting the rest of the building from potential aerosol leaks from risk areas as indicated in Figure 3 below. UV lamps will be installed at minimum 2,10 m highness from the floor. Safety in occupied areas will be assured if the level of radiation intensity is < 0,4 $\mu W/cm^2$. Their efficacy is proved by high UV light intensity at the level of the lamp (>100 $\mu W/cm^2$ at 1m from the lamp and >20 $\mu W/cm^2$ at 2 m distance from the lamp.

Fig 2: Louvered upper room UV fixtures, 254 nm, with horizontal flow of radiation, that may be placed on the lateral wall of the room

UV lamps provide additional protection against infectious aerosols. They should not be considered a substitute for a poorly functioning BSC or a BSC lacking certification.

Cleaning of the fixture is performed at least once each three months; UV lamp is cleaned with alcohol 70 % (no water or detergent).
Monitoring the efficiency of the UV light in the laboratory will be performed using the same UV-c meter recommended to be procured for the MDR TB isolation area
- UV light level (radiation intensity) is measured with a UV meter (254 nm) during installation process, then quarterly. Level of radiation has to be:

- < 0,4 uw/cm^2 at 1,70 m height, in any point of the room, proving safety for the person occupying the room) and
- > 100 uw/cm^2 at the height of the lamp, at 1 m distance from the lamp and > 20 uw/cm^2 at 2 m distance from the lamp, at same height, proving efficacy of the UV in the high room area.

- Changing of the UV lamp is performed after facing the lifetime of the lamp (18.000 hours) or when intensity of UV radiation is < 100 uW/cm^2 at 1 m from the lamp, measured at the height of the device.
Monitoring registration of the UV devices use shall be done by an hourly chart, which includes also the date of lamp cleaning.
UV lamps shall be included in the equipment service contract.

Figure 1. ***Mechanical ventilation system (HVAC) in risk areas, in addition to the biosafety cabinets***

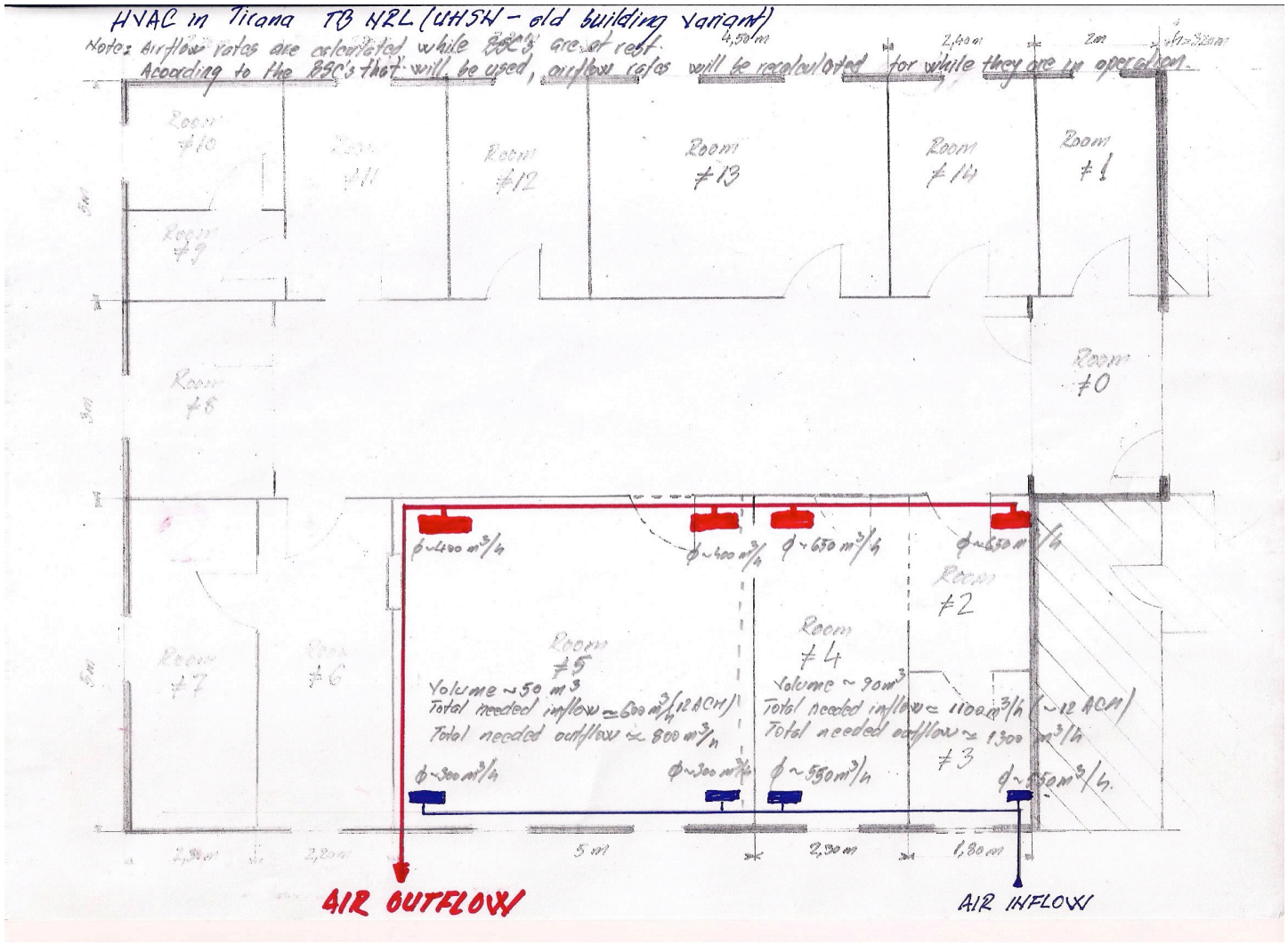


Figure 2: ***Proposed new layout of the renovated NRL***

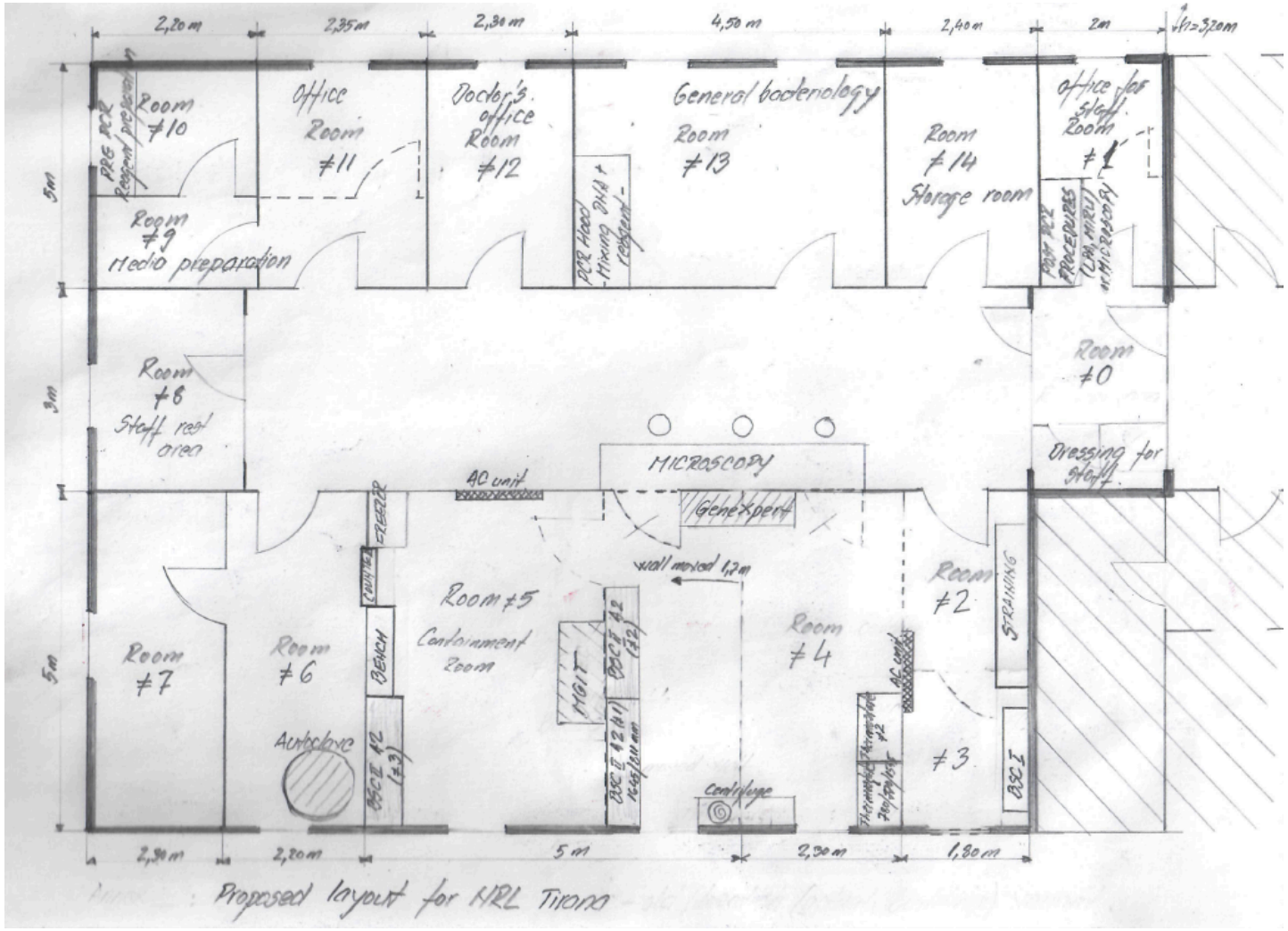
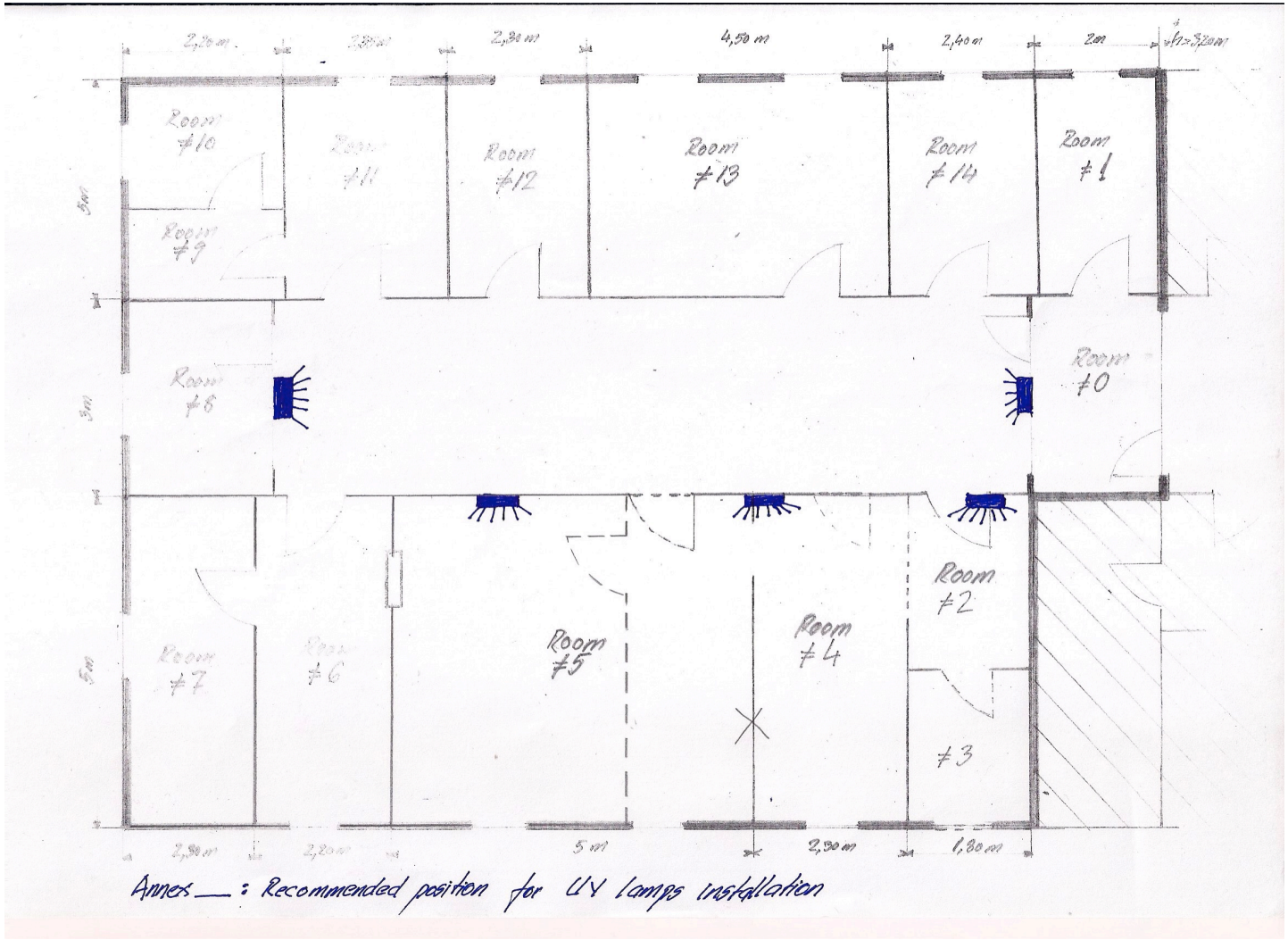


Figure 3. ***Layout of UV lamps installation in the laboratory***.



DELIVERABLE 3. List of new laboratory equipment to be procured during the lifetime of the Global Fund grant

Equipment at the NRL
Most of the equipment at the NRL needs replacement as indicated in Table 1 below. The Table indicates the current status of the equipment, the actual needs and whether replacement is included in the GF budget. Our recommendations are mentioned in the last column with related budget if not considered in the GF budget. We relied on the GF budget prepared on October 15, modules 7 "TB care and prevention" and module 9 "MDR-TB" where laboratory needs are addressed and copied in Table 2.
The second section of Table 1 shows the additional equipment required to meet the needs of either adoption of the new molecular tools for rapid detection of MDRTB or adjustment to the needs of increased activity related to the laboratory network strengthening.
According to our recommendations, an additional budget of 58 755 USD is needed to fulfil the equipment needs.

We have no comment regarding the consumable list, which covers all needs for microscopy, culture, DST by conventional and molecular (GenXpert) methods according to assumptions and indications of use made by the NRL in agreement with the NTP.

However, we would like to emphasize the following remarks:

- specifications of equipment and consumables should match the WHO recommendations on specifications for TB laboratories as detailed in the 2011 document "Guidance for countries on the specifications for managing TB laboratory equipments and supplies" available at apps.who.int/iris/bitstream/10665/44798/1/9789241503068_eng.pdf

- special attention should be drawn to the fact that the GF budget is based on FIND negotiated prices whereas Albania is on the list of eligible countries only for GenXpert.

- prices for equipment are usually given FOB (free on board) that means additional transport and shipping (international and local costs), insurance, clearing, customs duties and local taxes, where applicable, as well as the local representative costs are not included. It seems that for GenXpert only, additional FOB cost is at least partially considered with a travel cost of 1800 USD. However, additional FOB cost applies to all budget lines for equipment, including for the FIND-negotiated prices.

- there is no indication in the budget to cover maintenance, a critical issue considering the BSC. NRL BSCs are either out of order or have never been maintained since their installation in the laboratory. However, BSCs are the primary containment for the protection of personnel against infectious aerosols and preventing laboratory-acquired TB. The use of not well-maintained BSC is actually more hazardous than protective for the personnel. According to WHO recommendations, BSCs require annual maintenance to ensure proper functioning. Delaying maintenance or using under-qualified personnel to conduct maintenance can put laboratory workers at risk.

- the GF budget considers the purchase of HEPA filters for the 3 BSC that is of course necessary. However it should be stressed that procurement of new HEPA filters does not fit maintenance issues. BSCs once filters have been changed need to be certified. Certification with check of the functional and integrity of BSC have to be performed in line with

international performance standards by qualified service technicians. Tests include tests for HEPA filter leaks, assessments of the down flow velocity profile, face velocity, negative pressure and ventilation rate, airflow smoke pattern and alarm and interlocks.

- we strongly recommend the procurement of new class II type A2 BSC to replace the type B2 BSC currently available at the NRL as described in the deliverable 2. "Design of the mechanical ventilation of the NRL Tirana and its running costs". First, the advantages of the class II type A2 BSC are lower operating and maintenance costs, as they are re-circulating 70% of the air to the work area as the downward airflow. Air, heat and energy loss are not high with this model of BSC. On the contrary, certification, operation and maintenance of type B2 BSCs are more difficult and more costly. Second, class II type B2 BSC must be hard ducted. Therefore the building's exhaust system must precisely match the airflow requirements specified by the manufacturer for both volume and static pressure. By contrast, class II type A2 BSC must be ducted to the outside by canopy or Flex Air type connection. The major advantage of a thimble connected BSC is that no adjustments need to be made to the cabinet and the direction of air flowing from the laboratory to the outside will be maintained.
For all these reasons, WHO does not recommended the use of B2 BSC for any new TB laboratory.

- our recommendation for the renovated NRL (deliverable 1) implies the loss of the walk-in incubator to be replaced by incubator units. Based on the expected number of 6000 cultures per year, it is advisable to have 2 incubators of 400 litres each (usual dimensions WxDxH about 120x70x100). Incubators should be equipped of: i) shelves made of perforated stainless steel, usually not included with the incubator; ii) 20 trays especially designed for incubation of Lowenstein-Jensen tubes (150 mm), 20 tubes per tray (necessary at least for the first week of incubation).

- the GF budget covers the Y1 and Y2 needs of consumables for microscopy, culture and DST based on conventional phenotypic methods. For Year 3, the MOH is committed to identify adequate budget for ensuring the NRL needs to provide diagnostic services to all patients. It is a major challenge especially considering the current situation where shortages of culture and DST reagents/consumables prevail.

- benches, procurement and installation. Benches should be impervious to water, easy-to-clean, resistant to the chemicals and disinfectants normally used in the laboratory; location and length dimension of benches should be defined once main renovation is ended to get precise measurements of available space once internal walls are built. Similarly furniture in the laboratory should be sturdy, made of impervious materials and able to be decontaminated easily.

Table 1. List of equipment needed at the NRL

List of equipment currently available at the NRL

Current NRL equipment	Current status	Needs for:	GF Budget (USD)	Additional budget needed (USD)
Refrigerated Centrifuge	out of order	Refrigerated and aerosol-free centrifuge	7000	
Biosafety Cabinet Class II type B2 (3 units)	never maintained, one without any filter, no UPS	3 BSC class II type A2 plus 3 HEPA filters plus 3 UPS units plus maintenance	1845 for 3 UPS 4500 for HEPA filters	27000 for 3 BSC type A2 plus maintenance/annual certification
Biosafety Cabinet Class I	out of order			
BACTEC MGIT 960	proper functioning no UPS	maintenance contract plus UPS	0	1845 for UPS plus maintenance contract
Thermal Cycler 2720	already second hand when acquired	a last generation thermocycler	4595	
PCR Cabinet	proper functioning		0	
Twincubator	proper functioning		0	
Refrigerator with freezer compartment	proper functioning	1 freezer -20°C, 500 L 3 freezers -20°C, 90 L 1 refrigerator, 110 L 1 refrigerator 380 L for media preparation	5000 for 1 freezer -20°C	1020 (3 x 340 USD each freezer 90 L) 270 (1 refrigerator 110L) 1200 (1 refrigerator 380 L)
Fluorescent microscope	old generation not LED	3 LED FM microscopes	15640 for 1 LED microscope	18000 (3 x 6000 USD each Primostar LED microscope FIND) to replace the budget line of 15640
Light microscopes (3 pieces)	no possibility to use LED FM			
Steriliser	proper functioning acquired in 2000		0	
Incubators	one for General Microbiology		0	
Walk-in incubator room	Large capacity	2 incubators, 400 L capacity each, to replace the walk-in incubator room	0	12400 (2 x 6200 USD each incubator 400 L)
Autoclave 270 L	corroded, needs repair	1 for waste decontamination	10000	
Autoclave	suitable for surgical instruments not for waste management			
Distiller	out of order	one 4-liter/hour capacity distiller		2660
Centrifuge for molecular work	proper functioning		0	
TOTAL USD				74 395

We suggest to replace the item in blue by another equipment. Therefore, the additional cost may be reduced to 74 395 – 15 640 = **58 755 USD**

Additional equipment needs

Additional equipment	Cost per unit (USD)	Nb of units	In the GF budget (USD	Additional cost (USD)	Comment
GeneXpert IV-module with laptop Price	17 500	1	17 500		Procurement of rapid molecular tools for early diagnosis of TB and MDR TB, GenXpert machine/Y1
3 year warranty	7 900		7 900		
module calibration	1 800		1 800		
travel cost	1 800		1 800		
Genoextract	10 600	1	10 600		
Automated capillary electrophoresis analyser	30 000	1	30 000		Molecular typing for epidemiological purposes and contact tracing by MIRU-VNTR.
Kits and consumables	12 000	1	12 000		
Laboratory chairs with backrest	280	6	0	1 680	Adequate chairs for risk-areas: no wood, no cloth-covered, impervious material easy to decontaminate, comfortable for long work sessions
Vortex	220	6	0	1 320	One for each BSC and other benches to prevent move from a BSC to another place
Trolleys (side bench for BSC)	250	4	0	1 000	To avoid overloading of the BSC work area
Calibrated multi-channel pipette	1 000	1	0	1 000	For LPA and other molecular work
Calibrated variable volume pipettes	750	5	0	3 750	PCR-dedicated, DNA-free pipettes for PCR
Laminar flow cabinet (clean bench)	4 000	1		4 000	For preparation of PCR reagents free of contamination
TOTAL				**12 750**	

Table 2. Equipment and consumables for the NRL and the TB laboratory network as in the GF budget, Oct15, 2015

Infection control	UHSN ; Infective clinic; Pediatric clinic "Mother Teresa"; 12 UHSN ; Infective clinic; Pediatric clinic "Mother Teresa"; 12 regional hospitals; 9 laboratories; NRL In total 15 hospitals and 9 laboratories				Budget			Comments
Name of activity	**Name of product**	**Unit**	**Price per unit (USD)**	**Quantity**	**Y1**	**Y2**	**Y3**	
Capital investment	UV lamps	lamps	400	29	11 600			UV lamps for 15 hospitals and 9 laboratories
Capital investment	reparations, renovation		10000	3	30 000	0	0	In 3 hospitals will be investments on separation of rooms for TB patients
Capital investment	reparations, renovation		5000	3	15 000	0	0	Infrastructure Investments on 3 dispensaries
Recurrent expenses	masks (these are respirators)	unites per person, annually	3	4800	14 400	14 400	14 400	For 80 workers/ 60masks/person/year
Recurrent expenses	protective clothes (PPE)	unites per person, annually	10	4800	48 000	48 000	48 000	For 80 workers. We don't have unit price
Recurrent expenses	disinfectants	unites per facility, annually	20	24	480	480	480	For 24 facilities (15 hospitals and 9 laboratories)
Recurrent expenses	detergents	unites per facility, annually	20	24	480	480	480	For 24 facilities (15 hospitals and 9 laboratories)
Recurrent expenses	Betergents for washing the hands	unites per facility, annually	20	24	480	480	480	For 24 facilities (15 hospitals and 9 laboratories)
Recurrent expenses	aerosol equipment	unites per person, annually	54,7	20	1 094			For 20 facilities. Each facility one aerosol equipment. Please change the unit description to unit per facility/year/ The equipment to be procured only in Y1
Capital investment	LED microscopy	equipment	15640	1	15 640			
Capital investment	Autoclave	equipment	10000	1	10 000			
Capital investment	Centrifuge (refrigerated)		7000	1	7 000			
Capital investment	UPS (3) for Biosafety Cabinets NRL		1845	3	5 535			
Capital investment	HEPA filters	filters	1500	3	4 500			Maintenance of the laboratory equipment/ change of Hepa filters for biosafety cabinets /Y1
Capital investment	Freezer -20		5000	1	5 000			
Capital investment	Microscope binocular		6000	5	30 000			
Capital investment	Freezer -80		20000	1	20 000			
Capital investment	GTQ Cycler		4595	1	4 595			
Capital investment	Genoxtract		10600	1	10 600			
Capital investment	Automated capillary electrophoresis analyser.		30000	1	30 000			Molecular typing for epidemiological purposes and contact tracing by MIRU-VNTR. Procure equipment for automated capillary electrophoresis and necessary kits, reagents and consumables,
	Kits and consumables	1	12000	1	12 000	12 000	12 000	GF Y1, Y2 and Y3,
	IGRA tests		30	150	4 500	4 500	4 500	For immune-compromised cases (individuals with HIV, chronic renal failure, fibrotic lesions, silicosis, cancer head and neck, malnutrition, diabetes) 100 tests per year for UHCMT and 50 tests per year are anticipated where TST performs poorly (for UHSN).
	Procurement of tuberculin		200	80	16 000	16 000	16 000	

MDR TB					Y1	Y2	Y3	Comments
Catalog nr	**Name of product**	**Unit**	**Price per unit (USD)**	**Quantity**	**Total (USD)**	**Total (USD)**	**Total (USD)**	
	GeneXpert IV-module with laptopPrice		17500	1	17500			
	3 year warranty		7900		7900			
	module calibration		1800		1800			
	travel cost		1800		1800			
CE-GXMTB-RIF-10	XpertMTB/RIF, 10 tests	1kit/10 tests	99,80	200	19 960	19 960	19 960	

DELIVERABLE 4. ***Guidelines for airborne infection control***

On beside on the other measures to minimize risk of infection in the TB laboratory is the good microbiological technique; it is known that specialized laboratory equipment should be accompanied by, but can never replace appropriate procedures and good technical practice.

Good laboratory design and facilities or availability of the equipment designated to decrease the concentration of aerosols are also essential to ensure biosafety in the laboratory.

Airborne transmitted infection control measures in the laboratory should be stipulated in the infection control plan, a "code of practice", including all administrative, environmental and respiratory protection related regulations on airborne transmitted diseases;

Administrative controls are the first level of the hierarchy. These are management measures that are intended to reduce the risk or exposure to hazardous aerosols.
Administrative control measures consist of the following activities:

- Assigning responsibilities for TB infection control in the TB laboratory; the head of the laboratory is the coordinator of IC activities. He has to ensure that a biosafety management system is developed and adopted, as well as a safety or operation manual and a set of standard operating procedures; Each employee has it's own well-defined responsibilities that should be periodically revised and reminded.
- Personnel should be advised of special hazards and be required to follow standard practices and procedures.
- Periodically conducting a TB risk assessment of the laboratory; in addition to the annual risk assessment, it has to be performed when new procedures are implemented or new equipment purchased.
- Developing and implementing a written TB infection-control plan, containing all TB IC measures to be implemented A copy of most recent safety or operations manual with its date of issue should be available in the laboratory.
- Manuals explaining the procedures must be readily available in different parts of the laboratory. Procedures should be reviewed annually. Standard operating procedures should include details of risk assessments, and the mitigation and control measures identified and implemented.
- Ensuring the availability of recommended laboratory processing, testing, and reporting of results;
- Implementing effective work practices procedures
- Educating, training, and counselling health care workers on TB disease, including TB transmission;
- Referring for testing and evaluating workers who are at risk for exposure to TB disease; periodically screen for symptoms of TB disease. More attention to immunosuppressed workers, that should be advised and distributed to work in low risk areas
- Applying epidemiology-based prevention principles, including the use of setting-related TB infection-control data;
- Using posters and signs to remind proper IC practices, including cough etiquette (covering mouth when coughing) and respiratory hygiene;
- Limiting the access in the laboratory; the international biohazard warning symbol and sign must be displayed on the laboratory door; only authorized persons should be allowed to enter the laboratory's working areas by using limited access doors.
- ***Systems for heating, ventilation, air and containment (directional airflow, especially***

BSC) must have a permanent maintenance plan to ensure they always function properly.

- All procedures must be performed in such a way as to minimize or prevent the formation of aerosols and droplets. Contact transmission of infections (other than TB, that is airborne transmitted) should be also avoided:
  • Mouth pipetting must be strictly prohibited.
  • No materials should be placed in the mouth. All labels used in the laboratory must be self-adhesive.
  • The use of needles and syringes should be limited, and they should never be used as a substitute for pipetting.
  • Written documentation that may be removed from the laboratory must be protected from contamination.
  • All contaminated materials, specimens and cultures must be decontaminated appropriately before disposal or cleaning for reuse.
  • All accidents, spills and potential exposures to infectious materials must be reported to the laboratory manager. Records of such incidents and corrective actions taken need to be maintained for future prevention.
  • Standard operating procedure for handling accidents and spills must be developed and be available in the laboratory. Practical training must be provided at least annually to ensure the procedure is adopted and becomes an automatic response.
  • Packing and transportation of samples must follow applicable national or international regulations.
- Ensuring proper cleaning, sterilization, or disinfection of equipment that might be contaminated
- Eating, drinking, smoking, applying cosmetics and handling contact lenses are prohibited in the laboratory.
- Storing food or drink anywhere in the laboratory's working areas is prohibited.
- Open-toed footwear must not be worn in the laboratory.
- Assuring proper waste management:
  - Waste-management procedures must comply with all pertinent local or national requirements and regulations. Waste is anything that is to be discarded. The overriding principle in minimizing risks from waste is that all infectious materials should be decontaminated, incinerated, prepared to be buried or autoclaved. Discard bags should be used to segregate waste. Most glassware, instruments and laboratory clothing will be reused or recycled.
  - Separate autoclaves should be used to sterilize solutions or glassware (clean materials), and to decontaminate infectious materials. The following materials are suitable for autoclaving:
    • Instruments, glassware, media or solutions for sterile use in the general diagnostic TB laboratory;
    • Mycobacterial cultures for waste disposal;
    • All infectious materials from TB-containment laboratories
    • The time, temperature and pressure should be recorded each time the autoclave is run to monitor whether it is functioning properly. Biological indicators should be used regularly to validate the ability of the autoclave to achieve sterilization.

The second level of the hierarchy is the use of environmental controls to prevent the spread and reduce the concentration of infectious droplet nuclei. This includes two types of

environmental control.

- Primary environmental controls consist of controlling the potential source of infection by using local exhaust ventilation (e.g. biosafety cabinets, booths, exhaustion fans, hoods) and diluting and removing contaminated air by using general ventilation (natural or mechanical).
- Secondary environmental controls consist of cleaning the air by using high efficiency particulate air (HEPA) filtration, eventually ultraviolet germicidal irradiation.

Biological safety cabinets (BSC):

When properly maintained and used in conjunction with good laboratory techniques, BSCs provide effective primary containment for work with human pathogens. In containment levels 3 (as TB lab), all open vessel activities with infectious materials are conducted in a BSC. Every employee working in a BSC must be trained in its correct use and have a good understanding of the different types of cabinets and how they work

All processing and digestion of sputum samples and manipulation of liquefied sputum specimens must be conducted in a BSC. The cabinet is the primary form of containment while specimens are processed for culture inoculation or for performing direct DST. Hence, good microbiological techniques and proper use of the cabinet are critical to allow work to be conducted safely.

- Good microbiological techniques are essential to minimize the risk of aerosolization.
- Improper use of the cabinet allows aerosols to be released into the laboratory.
- BSCs should be seated away from thoroughfares and out of crosscurrents from doorways and air-inlet systems. Air expelled from properly maintained cabinets will have passed through HEPA filters at the top of the cabinet and so can be expelled either into the room or ducted to the outside, depending on the degree of sophistication of the ventilation system installed.
- The air curtain at the front of the cabinet is fragile and can easily be disrupted by people walking parallel to it, by open windows, air supply registers or laboratory equipment that creates air movement (e.g., vacuum pumps, centrifuges). BSCs should be installed in accordance with the requirements of the producer. They should be located away from high traffic areas, doors and air supply/exhaust grilles that may interrupt airflow patterns. A minimum unobstructed distance of 40 cm should be provided between the exhaust outlet on top of the cabinet and any overhead obstructions. Whenever possible, a 30 cm clearance should be provided on each side of the cabinet to allow for maintenance access. For ducted cabinets, blowers on the exhaust system should be located at the terminal end of the ductwork; failure of exhaust flow should signal an alarm to the user. To prevent pressurization of the cabinet, an interlock system should be installed to prevent the cabinet blower from operating whenever the exhaust flow is insufficient; an anti-backflow device to prevent reverse airflow through the HEPA filter may be required.
- Continuous operation of BSCs helps to control dust levels and other airborne particulates in the laboratory. If BSCs are operated only when needed in order to conserve energy, the balancing of laboratory room air must be considered. In some cases, room exhaust is balanced to include the air exhausted through ducted BSCs, and these cabinets must not be turned off.
- The provision of natural gas to BSCs is not recommended. Open flames in the BSC create turbulence, disrupt airflow patterns and can damage the HEPA filter. When

suitable alternatives (e.g., disposable sterile loops, micro-incinerators) are not possible, touch-plate micro burners that have a pilot light to provide a flame on demand may be used.

- The correct operation of BSCs must be verified before they are used and then annually, and after any repairs or relocation, in accordance with the field tests outlined in equipment's specifications. Moving a cabinet can cause damage to the HEPA filter and its seals. These tests include the downward velocity profile, the work access face velocity, the HEPA filter leak test and the airflow smoke patterns. Measuring and testing equipment must be calibrated and maintained. A copy of the certification report must be provided to the user and kept on file. A label indicating the date of certification, the date of the next certification, to what standard the tests were performed and the name of the certifier should be affixed to the exterior of the cabinet. Experienced qualified individuals must perform on-site field-testing

Follow these procedures for **working in the cabinet** *(according to the Tuberculosis Laboratory biosafety manual, 2012):*

- Don protective clothing and gloves as appropriate.
- Perform operations as far to the rear of the work area as possible.
- Avoid movement of materials or excessive movement of hands and arms through the front access opening during use; when you do enter or exit the cabinet, do so from straight on; allow the cabinet to stabilize before resuming work.
- Keep discarded, contaminated material to the rear of the cabinet; do not discard materials in containers outside of the cabinet.
- Do not work with open flames inside the cabinet.
- If there is a spill during use, surface decontaminate all objects in the cabinet; disinfect the working area of the cabinet while it is still in operation (do not turn the cabinet off).

Follow these procedures upon **completion of the work**:

- Allow the cabinet to run for 5 minutes with no activity.
- Close or cover open containers before removing them from the cabinet.
- Surfaces disinfect objects in contact with contaminated material before removal from the cabinet.
- Remove contaminated gloves and dispose of them as appropriate; wash hands.
- Don clean gloves, and ensure that all materials are placed into biohazard bags within the cabinet.
- Using a suitable non-corrosive disinfectant (e.g., 70% ethanol), disinfect interior surfaces of cabinet; periodically remove the work surface and disinfect the area beneath it (including the catch pan) and wipe the surface of the UV light with disinfectant.
- Turn off the fluorescent light and cabinet blower when appropriate (some cabinets must be left on at all times; if you are unsure, check with your cabinet certifier, safety officer or building maintenance personnel).
- Turn on the UV light if appropriate (do not turn on when people are working close by); UV must be tested to ensure that it is emitting a germicidal wavelength (ask your cabinet certifier to perform this test).

Ventilation *(according to the Tuberculosis Laboratory biosafety manual, 2012):*
In addition to the BSC (the primary barrier), the secondary barrier (provided by the

laboratory itself) is achieved by maintaining a unidirectional airflow into the laboratory, and by ensuring a minimum of 6–12 ACHs.
A simple means of creating unidirectional airflow is to place a vent that allows air to flow into the clean area of the laboratory and to operate continuously one or more thimble-fitted or hard ducted BSCs to draw air towards the dirty area, remove the air from the laboratory, and expel it outside the building. A visual monitoring device with or without an alarm should be installed so that staff can ensure at all times that proper directional airflow is maintained in the laboratory.
Ducting the BSC to the outside using a thimble connection helps create unidirectional airflow into the laboratory, and any contaminated air in the BSC is expelled from the laboratory through the HEPA filters in the BSC. When the cabinet is turned on, the external fan extracts air from both the cabinet and the room. When the cabinet is switched off, the expelled air will be extracted only from the room. An external fan can be installed with or without a link to the status (operating or stand-by) of the cabinet. It is best if the external fan has a separate switch from the BSC, or alternatively it can be coupled with a relay circuit so that the external fan continues operating for a given time after the BSC has been turned off to ensure that all of the air expelled from the BSC is vented outside. The major advantage of a thimble connected BSC is that no adjustments need to be made to the cabinet and the direction of air flowing from the laboratory to the outside will be maintained.
Alternatively, air expelled through the HEPA filters within the BSC can be released into the laboratory. However, in such cases, there must be a separate exhaust system for the building that ensures a minimum of 6–12 ACH in the laboratory. The building's ventilation system must be constructed in such a way that air from the moderate-risk laboratory is not recirculated to other areas within the building.
When air expelled from the laboratory is discharged to the outside of the building, it must be dispersed away from occupied buildings and air intakes.
Windows must be kept closed at all times in moderate-risk and high-risk TB laboratories.

Personal protective equipment *(according to the Tuberculosis Laboratory biosafety manual, 2012):*

The third level of the hierarchy of airborne IC is the use of **respiratory-protection control**. It consists of the use of personal protective equipment in situations that pose a high risk of exposure to infectious aerosols.
Use of respiratory protection equipment (FFP2 or FFP3 respirators) can further reduce risk for exposure of health care workers to infectious droplet nuclei that have been produced during high-risk procedures.
Respirators are not normally required for work in a TB laboratory. However, they may be recommended after a risk assessment if cultures are being manipulated within a TB-containment laboratory. Even if not worn regularly, respirators must be available in laboratories where culture manipulations are performed in case an accidental biohazard (such as a spill) occurs outside the BSC. Respirators should be included as part of a laboratory's spills clean-up kit.
Respirators should never be used as a substitute for a properly maintained and functioning BSC.
FFP2 (European Standard EN149:2001) or N95 (United States Standard NIOSH N95) respirators should be worn if indicated by a risk assessment.

If respirators are used in a laboratory, all staff should be instructed and trained in their proper use and fitting, and in their limitations. Ideally, staff should undergo a fit test to ensure leakage does not occur. Respirators must be stored in a convenient, clean, dry and sanitary location, and must not be worn outside of the laboratory. Once a respirator has been put on, under no circumstances should the wearer touch the front of it. Staff should not place the respirator under their chin or on their head when answering the phone or talking.
Respirators must be inspected before every use to ensure that there are no holes other than the punctures around the staples, and to ensure that no damage has occurred. (Enlarged holes resulting from ripped or torn filter material around staple punctures are considered to be damage.) Straps and valves must also be checked. A damaged respirator must be discarded and replaced immediately.

In addition to respiratory protection, protective laboratory clothing must be worn at all times while staff is working in the laboratory. Protective clothing must not be worn outside the laboratory area. Clean gowns and used gowns must be stored in different areas of the laboratory. Laboratory coats and gowns should be changed at last weakly, but laundering should not occur at home. Laboratory gowns should have long sleeves and elasticized cuffs (at least 30 mm long); they should fasten at the back. Different sizes of gowns should be available for staff. Gowns must be worn when working in a laboratory where there is a high risk of TB infection.
Laboratory coats usually have long sleeves and fasten in the front. Different sizes of laboratory coats should be available for staff.
Gloves must be worn for all procedures that involve direct contact, or may involve accidental contact, with sputum, blood, body fluids and other potentially infectious materials. After use, gloves should be removed aseptically and hands washed.
Personnel must wash their hands after any overt contamination, after completing work during which infectious materials were handled, and always before they leave the laboratory's working areas. Personnel should thoroughly lather their hands with soap, using friction, for at least 15 seconds; rinse them in clean water; and dry them using a clean paper towel. Automated or hands-free taps (faucets) are preferable. However, where these are not available, a paper towel should be used to turn off the tap to avoid recontamination of clean hands.

DELIVERABLE 5. Revised guidelines for TB laboratory investigation and strengthening the TB laboratory network

1. CASE DETECTION, DIAGNOSIS AND ORGANIZATION OF THE LABORATORY SERVICE

Background

Dispensaries of lung diseases

As a rule, General Practitioners should refer patients with respiratory complaints such as: cough or other types of complaints, when they are of the opinion that these patients need complementary, further examinations, to the pulmonologists, either in dispensaries or in the hospitals. But often patient themselves go to dispensaries without any recommendation for doing so.

In the instances when the physician of the dispensary either suspects or diagnosis the patient to have tuberculosis, he refers him/her for further hospital treatment in one of the three lung hospitals, to be extended to 12 hospitals including regional hospitals.

Status of the dispensaries

At the national level, there are two models for the organization of dispensaries. One part is a direct continuation of the structures of anti-TB dispensaries, with more than one pulmonologists, several nurses who used to be vaccinators, and one bacteriological laboratory; the other part has one physician and one or two nurses but no laboratory. Children who are tuberculosis patients receive treatment in the Mother Teresa University Hospital Centre, alongside with cases of meningitis or other extra pulmonary TB forms, according to the specializations.

Case Detection and Treatment

TB case detection in the dispensaries is based on clinical, X-ray (radiological) and bacteriological examination of direct sputum. As a rule, TB patients, and in particular those with pulmonary tuberculosis get hospitalized in one of the three lung diseases hospitals for the duration of the first two months, or rather until their conversion from sputum BK positive into sputum BK negative. After hospital discharge, treatment is given at dispensaries and active contact investigation is undertaken.

Implementation of the transportation system of samples from dispensaries to the NRL.

Organization

From every TB suspect in a dispensary, three consecutive sputum samples will be collected at the dispensary by the nurse.

At the dispensary, if there is a laboratory, direct smear examination will be performed; afterwards the same samples will be sent to the NRL in Tirana by a private courier company under contract. If there is no laboratory at the dispensary, the sputum samples will be sent directly to NRL.

- Sputum containers and UN 3373 packaging will be procured to dispensaries
- Sputum containers will be packed in UN 3373 packaging.
- Laboratory request forms filled by the clinician will be sent with the sputum samples of every patient.
- A log form will list all samples of the package

- The courier is contacted the day of collection of the 2nd/3rd sputum and asked to come to the dispensary within 48 hours
- The courier will deliver directly to the NRL on the same day as sputa are picked up at the dispensary
- Samples will be stored at +4°C while waiting to be transported
- A notebook will be used to record all incidents related to the transportation procedure (courier delays, courier performance, defects in documentation, improper packaging, etc) in every dispensary and at the NRL
- Incidents reported by dispensaries and the NRL will be transmitted to the courier manager to develop remedial action
- A responsible for the referral procedure will be nominated and trained at the NRL.
- The responsible person at each site should train a deputy to ensure continuity of services in case of absence.

Training of personnel involved

The training at the NRL will provide detailed instructions on how to organize the logistics (UN 3373 packaging, lab request forms, ZN results when available, log forms, contact with the courier, storage of containers at +4°C before their transportation, reports of the dates of sputum collection and dates of package transportation). It will also include training/refreshment training on how to teach patients to produce sputa, evaluation of quality of specimens and rejection criteria.

Courier delays of intervention: pick up and delivery of sputa

The courier has to come at the dispensary within 48 hours upon request and then has to reach the NRL the same day. It is critical for the success of the overall process that the NRL be reached in timely turnaround time, less than 7 days after sputum collection by the patient

N.B. It has to be stressed that the culture yield decreases drastically if the delays between the day of sample production by the patient and the day of sample processing at the NRL exceeds 7 days. This time limit is valid only for samples stored at +4°C during this period of time, if cold conditions are not maintained the delay is quite shorter.

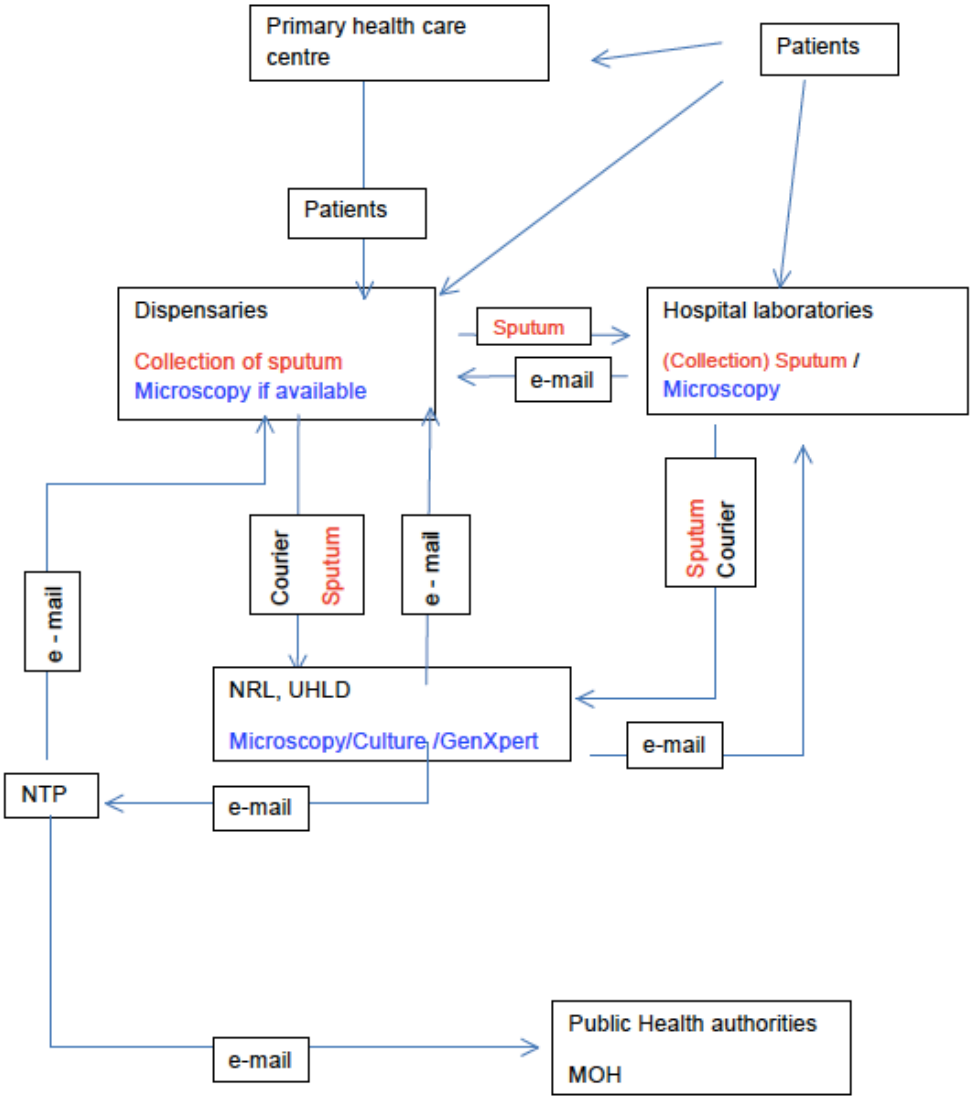
N.B. Cost of provision of courier services will be mainly based on calculations of kilometres to be covered by the courier. The number of TB cases detected in each dispensary per year will determine the expected number of passages per month or per quarter at each dispensary.

Bacteriological confirmation and reporting of TB cases

The NRL is responsible for the primary diagnosis of TB by GenXpert testing, culture, line probe assay (LPA) and drugs susceptibility testing (DST), as indicated in the scheme below. Implementing the GenXpert methodology, the NRL will follows the WHO recommendations for the rapid detection of MDR-TB patients. Moreover, the NRL has already the know-how and practice of LPA methodology and will be able to apply the most recent WHO recommendations issued in May 2016 for the detection of resistance to second-line drugs. The NRL reports results to the peripheral level and to the NTP. Positive detection of TB bacilli and/or detection of resistance to drugs will be reported immediately by Email or SMS to dispensaries to minimize delays before treatment. A definitive report of identification and DST will be sent by standard mail when available.

If the diagnosis is either given or confirmed in the lung diseases hospital, the dispensary is notified by the NTP.
The possibility for loosing/missing the reporting of cases with BK positive culture is almost inexistent as a double check is in place, between the NTP and dispensaries on the one hand, and between the NRL and dispensaries on the other.

Chart of organization of the transportation system and case detection



2. GUIDELINES FOR INTERNAL AND EXTERNAL QUALITY CONTROL

Microscopy

Workload:

maximum 20-25 ZN per technician per day, maximum 40-50 FM per technician per day

Maintenance of proficiency: at least 2-3 smears per day (10-15 smears per week)

Internal Quality Control (QC) of freshly made staining solutions

- Prepare batches of control smears from suitable sputum specimens. These are negatives that have been thoroughly examined, and a positive sputum homogenized after liquefaction by standing overnight at room temperature. Prepare at least 20 smears of each.
- Check every newly prepared staining solution with unstained control smears, using at least one positive and one negative slide.
- Examine the controls, and note the results in the QC logbook, under the batch number (and/or preparation date) of the new solutions.
- Unacceptable control results include the following:
  - AFB of positive controls are not stained strongly red or are too few in number.
  - Positive control background remains red or contains precipitates.
  - Negative control shows AFB (possibly from contaminated water).
  - Stain deposit is present on the QC slides.

  If one or more of these are found, discard the staining solutions and prepare new ones.

Internal QC of staining solutions and staining procedure

- Include positive and negative control smears with each day's reading.
- Proceed to staining
- Read control slides before patient smears.
- Unacceptable control results include the following:
  - AFB of positive controls are not stained strongly red or are too few in number.
  - Positive control background remains red or contains precipitates.
  - Negative control shows AFB (possibly from contaminated water).
  - Stain deposit is present on the QC slides.
- If results are unacceptable, re-stain smears of that day together with new controls, paying attention to correct technique; if these controls are also unacceptable, prepare new staining solutions and repeat the staining.

Internal QC indicators: operational indicators

Monitor laboratory performance by monthly counts – plotted on a graph – of:

- number of smears = workload
- **conversion rate** = number of follow-up patients with negative smears at 2-3 months / total number of follow-up patients examined
    - The conversion rate is a good **operational indicator** which shows the capacity of the NTP to maintain patients on treatment, obtain smear samples, and eliminate sources of infection
- **smear positivity rate** = number of positive smears /all number of smears
    - An increase in this indicator may represent changes in the population, or the reporting of false positive smears.
    - A decrease in this indicator may represent shifts in the population or the reporting of false negative smears.
    - Variation of the smear positivity rate can represent the need for training or re-training of staff.

The smear positivity rate provides an early warning of problems and signals the need for corrective actions.

Among the possible reasons for *false-positive results* are:

- re-use of containers or positive slides;
- contaminated stain prepared with water containing environmental mycobacteria;
- use of scratched slides;
- AFB floated off one slide and became attached to another during the staining procedure because there was no space between adjacent slides;
- inadequate decolourization;
- lack of experience, confusion with artefacts (especially if stains are not or poorly filtered);
- microscope (lamp) in poor condition or poorly adjusted: interpreting glitter as AFB;
- poor quality of staining solutions.

Among the possible reasons for *false-negative results* are:

- poor quality of specimen;
- not taking proper portion of specimen for smear preparation;
- excessive decolourization;
- poorly prepared staining solution;
- too little time staining with carbol fuchsin;
- over-staining with methylene blue;
- overheating during fixing;
- reading less than one length;
- slide exposed to daylight for too long;
- too long an interval between staining and reading, particularly if slides were poorly stained or not kept in the dark.

External quality control

External quality control methods for assuring quality of microscopy consist of on-site supervision, panel testing and blinded re-checking. Because the NRL will have to evaluate the performance of many sites, several among them new, and considering the scarce human resource available, it is recommended to perform EQA of microscopy based on the more simple method to implement, that means panel testing.

Panel testing ensures assessment of the testing capability of technicians for detecting, quantifying, interpreting and reporting AFB smear results using a set of prepared stained and un-stained sputum smears. The method identifies labs where serious deficiencies are present, allows evaluating technicians after they have completed training and monitors performance when resources are not available to conduct a blinded rechecking program.

Panels should contain at least 10 slides of stained and unstained smears, with a mix of smear positive and smear negative slides. Positive slides consist of differing grades of positivity, tending towards scanty and 1+ grades

Panels of specimens are sent to multiple test sites by the NRL. Test sites perform tests and report results. Results are often compared across several testing sites. They indicate quality of personnel performance and test site operations.

Culture:

Evaluation of culture performance is based on operational indicators. There is no applicable external quality assurance.

Workload

Culture (liquid/solid media): 20-40 specimens processed per technician per day

Proficiency

For maintaining proficiency in culture, laboratories should process a minimum of 20 specimens per week with a minimum of 5 cultures per technician.

Determination of the contamination rate: indicator based on the number of tubes/vials inoculated

The contamination rate is a valuable indicator of the efficiency of procedures used for specimen processing. It is calculated as the percentage of contaminated tubes among all inoculated tubes or vials and not as the percentage of samples. It should be within the range 2–5% with use of solid media. Using liquid media, the percentage contamination is usually higher but should not exceed 10%; otherwise the contribution of culture to diagnosis is not cost-effective.

Operational indicators based on the number of specimens processed

Depending on the workload and the prevalence of bacteriologically positive cases, analysis of the results obtained during a month, a quarter or a semester allows the detection of systematic errors. These analyses are key to the quality control of diagnostic cultures of pulmonary TB in adults (but do not apply to follow-up).

Classification of specimens from adult pulmonary TB patients investigated for diagnosis:

a smear-positive and culture-positive
b smear-positive and culture not done
c smear-negative and culture-positive
d smear-positive and culture-negative
e smear-positive and culture contaminated
f smear not done and culture-positive

From this classification, calculate the following indicators:

- Contribution of culture to diagnosis over microscopy

$$\frac{c}{a + c + d + e} \times 100$$

Culture is more sensitive than smear microscopy and is expected to contribute at least 20% to the bacteriological confirmation of adult pulmonary TB cases.

- Percentage of smear-positive and culture-negative diagnostic cases

$$\frac{d}{a + c + d + e} \times 100$$

This percentage should be very low, typically around 2-3 %. Exceptionally, patients are found with persistent smear-positive and culture-negative diagnostic specimens. These are usually undisclosed treatment control specimens. Higher percentages could be the result of decontamination procedures that are too harsh or of transport delays.

The indicators in the following table are valid for specimens from adult pulmonary TB patients investigated for diagnosis (no children, no extra-pulmonary specimens, no treatment follow-up):

Indicators of culture performance	Normal value (%)	Much higher: investigate	Much lower: investigate
Contribution of culture to bacteriological diagnosis of tuberculosis (based on **specimens**)	20	A	B and C
Percentage of smear positive/culture negative **specimens**	2–3	C and D	Not a problem
Percentage of contaminated **tubes**	2–4 6–8 with liquid media	E	F

A	• Smear microscopy reading errors: false-negatives" • A high percentage of incipient pulmonary TB and paediatric TB cases are being tested (not a problem)
B	• Inadequate use of culture: patients who are not TB suspects are being examined, rather than incipient TB cases
C	• Excessive delay between specimen collection and specimen processing • Over-harsh specimen decontamination procedures (excessive concentration and/or too long a contact time with the decontaminant) • Low relative centrifugal force or overheating of centrifuge • Low culture media sensitivity (lack of homogeneity, overheating during inspissations, too much malachite green, too acidic a pH) • Incubation at too high or too variable a temperature • Misclassification of a follow-up specimen
D	• Smear microscopy reading errors: false-positives
E	• Un-refrigerated storage of specimens • Excessive delay between collection and processing of specimens • Low decontaminant concentration • Too short a contact time between decontaminant and specimen • Deficiency in the sterilization procedure • Careless use of the Bunsen burner, heavy people movement in the work area, generation of air draughts by fans or air-conditioning systems, etc,
F	• Too high a concentration of decontaminant • Too long a contact time of the specimen with the decontaminant • Poor specimen neutralization • Too high a concentration of malachite green in the culture medium • Incubation at too high or too variable a temperature

Other alarms to check

Recurrent contamination

Recurrent contamination can occur in specimens processed during a particular day or in decontaminated specimens or in specimens collected in one particular place. In such cases, the sterility of the decontamination reagent solutions, of the whole decontamination process or of the specimen collection/transportation system will have to be checked; if errors are detected, immediate remedial action must be implemented. If the problem is traced to the technologist performing the procedure, he or she should be immediately retrained.

If contamination of specimens from the same patient recurs, a harsher decontamination procedure may have to be used for further specimens from the patient. Increase the reagent concentration, *not* the time of exposure. Use two volumes of decontaminant solution to one volume of specimen. Do not apply the modified procedure to all specimens – only to contaminated specimens.

Clustering of culture-positive specimens

Cross-contamination between specimens from epidemiologically unrelated patients could cause a sequence of positive culture isolations in a short interval of time. The occurrence of cross-contamination should be investigated to rule out false-positive culture diagnoses. The following circumstances will be investigated:

- some of the patients involved do not have clinical symptoms compatible with tuberculosis;
- other specimens from the same patient are not culture-positive;
- one or several specimens involved, which yielded cultures with very few colonies, were processed immediately after a highly smear-positive specimen.

If cross-contamination is likely, the cultures should be studied by genotyping.

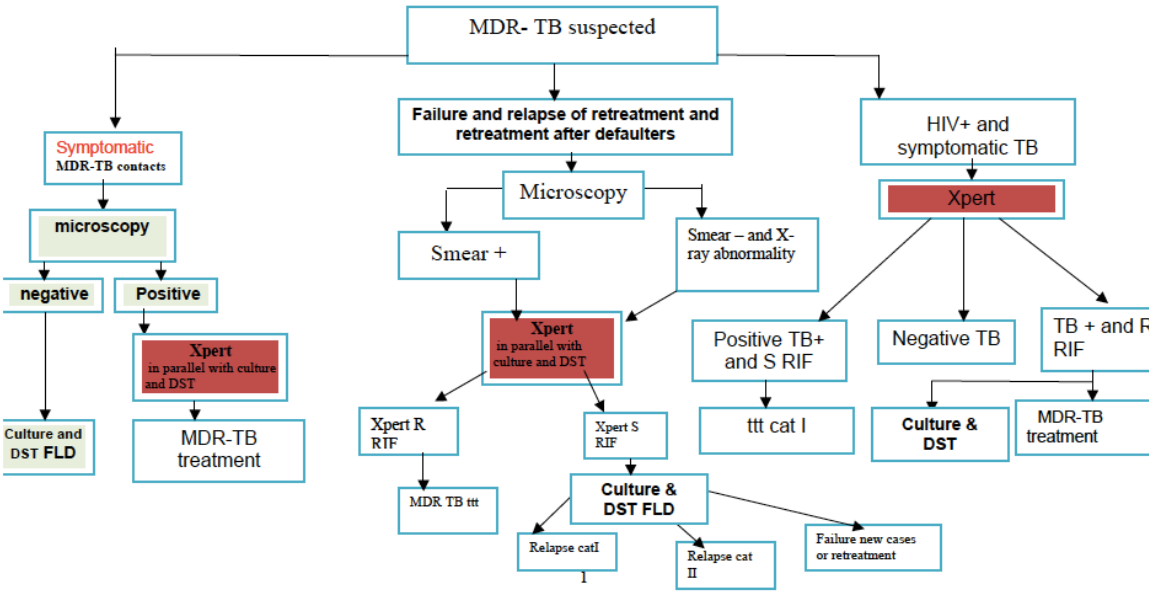
If cross-contamination cannot be ruled out, check that the following precautions are being respected:

- solutions are dispensed without touching the necks of the tubes;
- aliquoted reagent solutions are being discarded after single use;
- the processing sequence of specimens is maintained, i.e. smear positive specimens are processed last;
- tubes are not uncapped simultaneously or immediately after being taken from the centrifuge;
- supernatants are discarded carefully;
- gloves, if worn, are frequently changed and never reused.

It may be advisable to have a BSC dedicated to processing of smear-positive specimens or to process all positive specimens after the negative ones since the probability of cross-contamination increases with the number of smear-positive specimens.

3. DIAGNOSTIC ALGORITHMS TO BE USED AT THE NRL WHEN GENXPERT WILL BE AVAILABLE

1. Use of GenXpert as the initial or follow-on diagnostic test as illustrated in the scheme below for:
   - suspected MDR-TB cases including MDR-TB contacts, failure and relapse of retreatment and retreatment
   - HIV-positive patients



2. Use of GenXpert as the initial diagnostic test in all children suspected of having TB
3. Use of GenXpert as the initial diagnostic test for CSF specimens from patients suspected of having TB meningitis and for testing other non-respiratory specimens (lymphnodes and other tissues) from patients suspected of having extra-pulmonary TB.
4. Use of GenXpert as the initial diagnostic test in smear-negative patients highly suspected of having TB
5. Use of GenXpert as the initial diagnostic test in contact of sputum smear-positive cases
6. Use of GenXpert as a follow-on diagnostic test in all sputum smear-positive cases