The Tuberculosis Laboratory Initiative

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Executive Summary

Infectious pulmonary tuberculosis (TB) is accurately diagnosed by microscopic examination of respiratory specimens. Laboratories that provide reliable and timely microscopy results are thus the cornerstones of an effective TB control program. Traditionally respiratory specimens were neither homogenised (increases diagnostic reliability) nor concentrated (increases diagnostic sensitivity) and were examined using a low-contrast stain (Ziehl-Neelson) at the limits of light-microscope detection. This procedure is relatively low-cost but is not as effective or reliable as the procedures applied by up-to-date TB laboratories (homogenization, concentration and high-contrast [fluorescent] microscopy). The STOP TB LVIV Project Group thus proposes the improvement of the laboratory detection of infectious tuberculosis in the Lviv oblast of Ukraine by putting into effect the **concept of laboratory modules**.

Introduction

The mycobacteriology laboratory is at the heart of tuberculosis (TB) control programs; a TB control program that does not invest in laboratory staff, procedures and equipment is bound to fail in the end. The STOP TB LVIV Project Group therefore proposes that a step-wise laboratory initiative be launched to improve the performance of the existing mycobacteriology laboratories of the Lviv oblast of Ukraine.

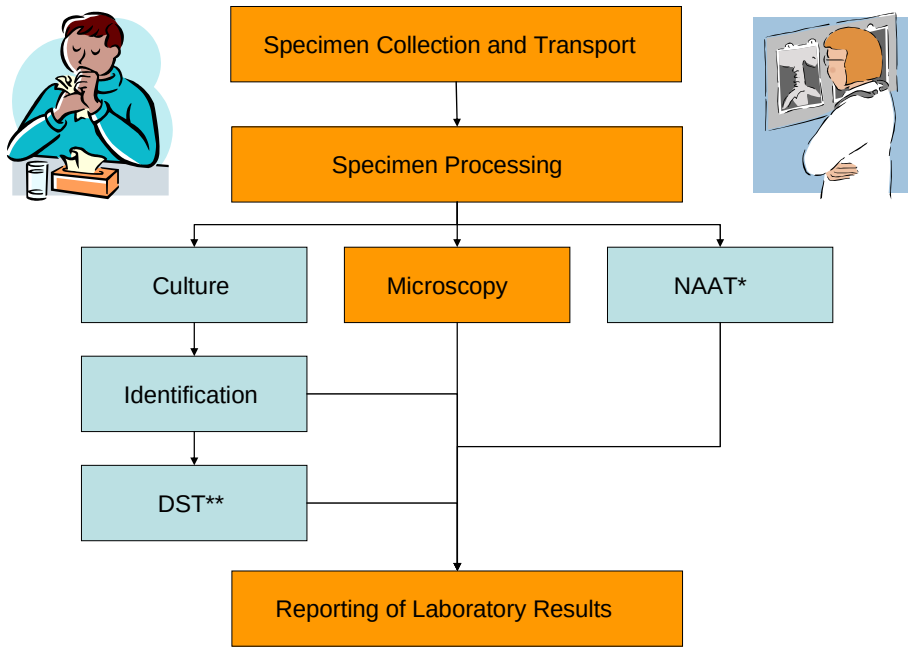
In order to achieve the best added-value-for-money the individual stages (functions) of the diagnostic process were identified. Based on the principle “form follows function” this approach guaranties the most effective allocation of scarce resources.

The **Core Laboratory** is essential to the diagnosis of an infectious case of TB and spans the entire diagnostic process, including the pre-test and post-test phases. Its functional modules are specimen collection and transportation, specimen processing, microscopy, and reporting of laboratory results (figure 1, orange boxes).

The Core Laboratory can be upgraded by the addition of other modules, depending on the local level of service required by the laboratory network of the TB control program (1). The full implementation of all modules will be reserved for the central laboratory; any change in future service requirements can be made quite easily because the service is based on the “functional modules” approach.

The additional modules are: the **Culturing Module**, the **Identification (ID) Module**, the **Drug Susceptibility Testing (DST) Module**, and the **Nucleic Acid Amplification Techniques (NAAT) Module** (figure 1). NAAT can be applied either directly to processed clinical specimens or can be used to complement the methods of the ID and DST Unit, respectively.

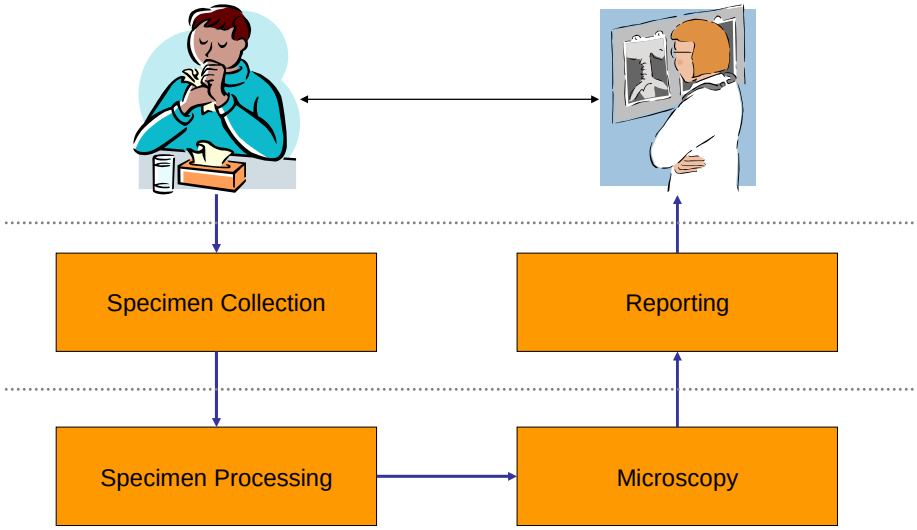
Figure 1 - The module concept



The Core Laboratory

The Core Laboratory is essential for the diagnosis of an infectious case of TB and spans the entire diagnostic process, including the pre-test and post-test phases. Its modules are specimen collection and transportation, specimen processing, microscopy, and reporting of laboratory results (figure 2).

Figure 2 - Modules required for a Core Laboratory



Specimen Collection and Transportation

The period from the collection of the clinical specimen to its acceptance by the laboratory is designated the **pre-test phase**. Here, many important decisions are taken that may have a substantial impact on the quality of the laboratory results. Thus, the laboratory must define for clinicians the requirements of collection, storage and transportation in order to ensure that the specimens arrive at the laboratory in the best possible condition.

The requirements for collection, storage and transportation are on the **request form** that comes with every specimen and covers diverse issues, such as:

- Identification of the patient
- Clinical indication (e.g. new cases only, no chronic cases)
- Type of specimen
- Minimal amount of specimen
- Maximal number of specimens per patient and episode
- Transport of specimens (container, storage, temperature)
- Rejection criteria

Investment

Equipment, procedures (SOP[1]) and disposables are detailed in **addendum A**.

Specimen Processing

The **test-phase** consists of the modules specimen processing and microscopy. In order to systematically monitor the quality of the testing phase the participation in an external quality assessment (EQA) scheme, e.g. UK NEQUAS, is highly recommended.

Upon arrival in the laboratory specimen and request form are checked for inconsistencies. In addition, the suitability of the specimen for testing is confirmed on the basis of the rejection criteria. Thereafter, the specimen is registered and processed according to the SOP.

Investment

Equipment, SOPs and disposables are detailed in **addendum B**.

Fluorescence Microscopy of Processed Specimens

Processed specimens are applied to glass microscope slides and are then stained with the fluorochrome Auramin-O (SOP) and inspected using an LED-based microscope (SOP). Microscopy results are given as a score.

Investment

Equipment, SOPs and disposables are detailed in **addendum C**.

Reporting of Laboratory Results

The **post-test phase** consists of the authorisation of reports and the reporting of the results to the physician in charge of the patient. Periodic reporting to the Public Health Authorities is a part of this phase.

The regular review of the laboratory results by the laboratory supervisor will help to identify potential for continuous improvement of the laboratory's performance.

Investment

Equipment, SOPs and disposables are detailed in **addendum D**.

[1] Standard Operating Procedure

The implementation plan

General considerations

The change to the concept of laboratory modules will be started off by establishing a Core Laboratory within the existing premises of the central TB laboratory at Sychiv.

From there, Core Laboratories can be duplicated and distributed as specified by the TB control program in order to establish an Oblast-wide network of Core Laboratories. The staff of the central TB laboratory at Sychiv will be responsible for the supervision of the existing and for the implementation of new Core Laboratories.

After its successful implementation at Sychiv the local Core Laboratory can be up-graded by adding modules according to the priorities of the TB control program (1).

The Key Factors of Success

The successful change to the concept of laboratory modules depends on three key factors:

- the availability of highly motivated and well-trained laboratory staff
- the successful implementation of new laboratory work-flows and procedures
- sufficient funding (see below)

Laboratory Staff

Laboratory staff needs to be trained in order to achieve the high level of skills required for the successful change to the Core Laboratory technology and procedures. The STOP TB LVIV Project Group can provide training at the Mycobacteriology Laboratory of the Institute for Infectious Diseases, University of Berne, Switzerland. In order to launch the Tuberculosis Laboratory Initiative we propose a comparison of the new alternative method to the existing laboratory procedures (reference) during a pilot study (2).

New Laboratory Work-Flows and Processes

The SOPs of the Mycobacteriology Laboratory, Institute for Infectious Diseases, Berne will be adapted and will form the basis of the training in Berne. Following translation they will be used without further modification in the Oblast network of core mycobacteriology laboratories.

Investment

As specified in the addenda A to D

References

1 Bodmer T., *et al*. Proposal for a Mycobacteriology Reference Laboratory Service for the Oblast of Lviv, Ukraine (draft version)

2 Bodmer T. & J.-P. Zellweger. Detecting New Cases of Infectious Pulmonary Tuberculosis. A Pilot Study for the Evaluation of Two Diagnostic Procedures.

Addendum A

The Core Unit – Specimen Collection

Equipment

None

Procedures

Create a request form that meets the criteria given above

Create and distribute flyers for clinicians (explanation of rejection criteria)

Disposables

Containers for the collection and transport of clinical specimens

Item	Number	Price	Sum
Specimen collection tubes (50 mL), 360 tubes	1	€ 73.20	€ 73.20
Transportation containers, 300 units	1	€ 103.00	€ 103.00
Total			*€ 176.20*

Addendum B

The Core Unit – Specimen Processing

Equipment

Item	Number	Price	Sum
Bio-safety cabinet, class IIA	1	€ 10'900.00	€ 10'900.00
High-speed centrifuge	1	€ 7'445.00	€ 7'445.00
Rotor for centrifuge	1	€ 2'148.00	€ 2'148.00
Vortex	1	€ 234.00	€ 234.00
Refrigerator, 86 L	1	€ 455.40	€ 455.40
Dispensor, 50 mL	3	€ 299.55	€ 898.65
Dispensor, 5 mL	7	€ 197.85	€ 1'384.95
Racks for 50 mL tubes	10	€ 15.55	€ 155.50
Glass bottles, 1 L, 10 units	1	€ 52.10	€ 52.10
Glass bottles, 500 mL, 10 units	1	€ 37.20	€ 37.20
Total			*€ 23'710.80*

Procedures

N-Acetyl-L-Cystein Decontamination Procedure (SOP)

Disposables

Item	Number	Price	Sum
Centrifugation tubes, 50 mL, 360 tubes	1	€ 73.20	€ 73.20
Decontamination solution BD, 10 x 75 mL	1	€ 67.00	€ 67.00
Benchkote, 460mm x 50 m	1	€ 77.50	€ 77.50
Disinfectants (Lysoformin), 5 L	1	€ 34.70	€ 34.70
Waste container, 3 L	6	€ 11.50	€ 69.00
Waste container with lid	2	€ 20.00	€ 40.00
Bags for autoclave, 120x180 mm, 100 units	1	€ 4.80	€ 4.80
Bags for autoclave, 600x780 mm, 500 units	1	€ 177.45	€ 177.45
Gloves, 100 units per box	1	€ 5.00	€ 5.00
UK NEQUAS EQA scheme			
Total			*€ 548.65*

Addendum C

The Core Unit – Fluorescent Microscopy of Processed Specimens

Equipment

Item	Number	Price	Sum
Partec CyScope TB, fully equipped*	1	€ 1'680.00	€ 1'680.00
USB CCD Colour Camera*	1	€ 390.00	€ 390.00
Freight charges to Berne, Switzerland*	1	€ 45.00	€ 45.00
Laptop computer	1	€ 750.00	€ 750.00
Staining rack/bench	1	€ 44.80	€ 44.80
Total			*€ 2'909.80*

*as offered (Quotation No. 08010181)

Procedures

Auramin-O staining procedure (SOP)

Microscopy (SOP) and scoring form

Microscope maintenance (SOP)

Disposables

Item	Number	Price	Sum
Auramin-O staining solution, BD kit	1	€ 98.60	€ 98.60
Glass slides, 72 slides per box	1	€ 42.40	€ 42.40
Washing bottles, 500 mL	6	€ 2.25	€ 13.50
Transfer pipettes, 3.5 mL, sterile, 840 units	1	€ 68.90	€ 68.90
Waste container			
Total			*€ 223.40*

Addendum D

The Core Unit – Reporting of Laboratory Results

Equipment requirements

Computer, software and printer?

Procedures

Report authorisation (SOP)

Statistics for the Public Health Authorities (SOP)

Laboratory review (SOP)

Disposables

None