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LABORATORY SERVICES *in* TUBERCULOSIS CONTROL

PART I: ORGANIZATION AND MANAGEMENT

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ORGANIZATION AND MANAGEMENT

PART I

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PREFACE

Within the framework of National Tuberculosis Programmes the first purpose of bacteriological services is to detect infectious cases of pulmonary tuberculosis, monitor treatment progress and document cure at the end of treatment by means of microscopic examination. The second purpose of bacteriological services is to contribute to the diagnosis of cases of pulmonary and extra-pulmonary tuberculosis.

Standardisation of the basic techniques for tuberculosis bacteriology has so many advantages that it has become a necessity. The absence of standardised techniques complicates the activities of new laboratory services as well as the organization of existing laboratories into an inter-related network. Standardisation makes it possible to obtain comparable results throughout a country; it facilitates staff training, delegation of responsibilities and the selection of equipment, materials and reagents to be purchased; it also facilitates the evaluation of performance and the establishment of suitable supervision in order to increase efficiency and reduce operational costs.

Standardised techniques and procedures are useful if they meet the needs of - and are prepared in accordance with - prevailing epidemiological conditions and different laboratory levels. These techniques should be simple (to obtain the widest coverage) and should be applicable by auxiliary laboratory workers. At the same time, their sensitivity and specificity must guarantee the reliability of results obtained.

While tuberculosis laboratory services form an essential component of the DOTS* strategy for National Tuberculosis Programmes, they are often the most neglected component of these programmes. Furthermore, the escalation of tuberculosis world-wide, driven by the HIV epidemic and aggravated by the emergence of multidrug-resistance, has resulted in renewed concern about safety and quality assurance in tuberculosis laboratories.

The above considerations have led to the preparation of guidelines for laboratory services for the framework of National Tuberculosis Programmes. These guidelines are contained in a series of three manuals, two of which are focused on the technical aspects of tuberculosis microscopy and culture and a third which deals with laboratory management, including aspects such as laboratory safety and proficiency testing. These manuals have been developed for use in low-and middle-income countries with high tuberculosis prevalence and incidence rates. Not only are they targeted to everyday laboratory use, but also for incorporation in teaching and training of laboratory and other health care staff.

Finally, in order to adapt the functioning of bacteriological laboratories to the needs of integrated tuberculosis control programmes, information on control programme activities has been included. It is hoped that the series on laboratory services will enable National Tuberculosis Programmes to draw up national laboratory guidelines as one of their essential components.

**Directly Observed Treatment, Short course (The "brand name" for the WHO recommended strategy for tuberculosis control).*

1 INTRODUCTION

Tuberculosis bacteriology is one of the fundamental aspects of a national tuberculosis control programme and a key component of the DOTS strategy, yet the tuberculosis laboratory service is often the most neglected component of these programmes.

Diagnosis of tuberculosis and monitoring of treatment progress rely heavily on bacteriological examination of clinical specimens. The usefulness, priority and scope of the various techniques used in tuberculosis bacteriology depend on the epidemiological situation prevailing in individual countries and on the resources available.

1.1 Microscopy

Despite recent advances in mycobacteriology, early laboratory diagnosis of tuberculosis still relies on the examination of stained smears. Microscopy of sputum smears makes a particularly important contribution since the technique is simple, inexpensive and detects those cases of pulmonary tuberculosis who are infectious, ie. those responsible for maintaining the tuberculosis epidemic. Currently no other diagnostic tool is available which could be implemented affordably.

Smear sensitivity (diagnostic sensitivity of smear):* Direct smear microscopy using acid-fast stains is generally considered to be a relatively insensitive diagnostic procedure, with the reported sensitivity ranging from 25% to 65% when compared to culture. What is not generally appreciated, however, is that smear sensitivity varies with the type of lesion, the type and number of specimens, the mycobacterial species, staining technique and the alertness and persistence of the microscopist. In addition, the above sensitivity refers to bacteriological diagnosis of pulmonary TB, but smear examination identifies the cases which are sources of infection to the community, with a sensitivity of approximately 90%.

The minimum number of acid-fast bacilli necessary to produce a positive smear result has been estimated to be between 5 000 and 10 000 per millilitre (Table 1). Sputum specimens from patients with pulmonary tuberculosis - particularly those with cavitary disease - often contain sufficiently large numbers of acid-fast bacilli to be readily detected by direct microscopy. The sensitivity can further be improved by examination of more than one smear from a patient. Many studies have shown that examination of two smears will on average detect more than 90% of infectious tuberculosis cases, both in low- and high-prevalence countries. The incremental yield of acid-fast bacilli from serial smear examinations has been shown to be 80%-82% from the first, 10-14% from the second and 5-8% from the third examination.

**Sensitivity = Capacity of a diagnostic test to distinguish correctly in a population those individuals who have the disease, ie. the true positives*

Table 1. Numbers of acid-fast bacilli observed in smears, concentrations of culturable bacilli in sputum specimens and probability of positive results[1]

NUMBER OF BACILLI OBSERVED	ESTIMATED CONCENTRATION OF BACILLI PER ML SPUTUM	PROBABILITY FOR A POSITIVE RESULT
0 in 100 or more fields	less than 1 000	less than 10%
1-2 in 300 fields	5 000-10 000	50%
1-9 in 100 fields	about 30 000	80%
1-9 in 10 fields	about 50 000	90%
1-9 per field	about 100 000	96.2%
10 or more per field	about 500 000	99.95%

Considering the type of specimen, overnight sputum is better than sputum collected on the spot; for operational reasons, however, it is often necessary to depend on both. The recommended policy for sputum collection for case-finding by microscopy is, therefore, as follows:

- one spot specimen when the patient first presents to the health service
- one early morning specimen (preferably the next day)
- one spot specimen when the early morning specimen is submitted for examination

Smear sensitivity is very low in extrapulmonary and childhood tuberculosis and in most cases of disease caused by mycobacteria other than tubercle (MOTT) bacilli. In high-prevalence countries these conditions are, however, far less common than pulmonary tuberculosis and are not rapidly progressive or highly infectious. From a public health perspective, infectious pulmonary cases have the highest priority for detection and treatment until cure.

Smear specificity (diagnostic specificity of smear)*: When discussing the specificity of acid-fast smears, a distinction must be made between those instances where the specimen truly does not contain tubercle bacilli (ie. false-positive smears) and those where tubercle bacilli present in the specimen fail to grow in culture (ie. false-negative cultures).

[1] *David HL. Bacteriology of mycobacterioses. US Department of Health, Education and Welfare, Public Health Service, Communicable Disease Centre, Atlanta, USA, 1996.*

**Specificity = Capacity of a diagnostic test to distinguish correctly in a population those individuals who do not have the disease, ie. the true negatives*

When false-negative cultures are excluded, a residual number of genuine false-positive smear remain. Direct smear microscopy for diagnosis of acid-fast bacilli has a specificity of more than 98%. The preventable causes of false-positive smears are multiple: Mycobacteria often contaminate the water or solutions used in staining, while transfer of acid-fast organisms from positive to negative smears may occur during the staining process or via microscope immersion oil or objective lenses. False positive smears have also been reported to result from failure to properly filter and regularly replace reagents, from staining of artifacts present in the smear, from insufficient de-staining of non-acid-fast organisms and from retention of acid-fast stain by nonmycobacterial organisms.

Auramine staining procedures may produce a higher false-positive rate than carbolfuchsin methods, presumably because auramine may stain inanimate objects. Studies conducted in laboratories in Africa showed that about 1% of false positive smears could be attributed to transfer of organisms and another 1% to various administrative errors.

Careful quality control of microscopy procedures and review of positive smears can markedly decrease the incidence of false-positive smears.

Predictive value:* Sensitivity and specificity are not the only determinants of the value of microscopy as a diagnostic test. Microscopy can produce results of varying accuracy in varying epidemiological situations, even though the sensitivity and specificity remain constant.

The prevalence of tuberculosis has a decisive influence on the value of microscopy as measured by the predictive value: a high predictive value (90% or more) can be achieved when the prevalence of tuberculosis in the population tested is 10% or more. With such prevalence rates, high predictive values can be obtained with a test of high specificity, even if its sensitivity is low (as is the case with acid-fast microscopy). The prevalence of tuberculosis among adult patients attending health centres with complaints of prolonged chest symptoms in high prevalence countries is usually in the order of 10%, which provides the basis for microscopy as a good diagnostic test for tuberculosis in these countries.

Systematic investigation of respiratory symptoms (cough > 3 weeks) by smear examination of Sputum specimens in adult patients who consult primary health care services is the priority for tuberculosis case-finding

Most patients with infectious tuberculosis have respiratory symptoms and the use of smear microscopy in those presenting to health services with suggestive symptoms constitutes the most efficient means of case detection.

**Predictive value = Probability of having the disease among those classified as positive* (positive predictive value) *or probability of those not having the disease among those classified as negative* (negative predictive value)

1.2 Culture

Examination by mycobacterial culture provides the only definitive diagnosis of tuberculosis. However, the usual microbiological techniques of plating clinical material on selective or differential culture media and subculturing to obtain pure cultures cannot be applied to tuberculosis bacteriology. Compared with other bacteria which typically reproduce within minutes, *Mycobacterium tuberculosis* proliferate extremely slowly (generation time 18-24 hours). Furthermore, growth requirements are such that it will not grow on primary isolation on simple chemically defined media. The only media which allow abundant growth of *M. tuberculosis* are egg-enriched media containing glycerol and asparagine Lowenstein Jensen (LJ), and agar/liquid media supplemented with serum or bovine albumin.

Depending on the type of culture medium and decontamination method used, as few as ten viable bacilli can be detected. Culture increases the number of tuberculosis cases found, often by 30-50% and detects cases earlier, often before they become infectious.

If specimens from non-sterile body sites are not decontaminated, tubercle bacilli will easily be overgrown by more rapidly dividing organisms, eg. bacteria and fungi. Selective decontamination of specimens is, therefore, required to destroy rapidly growing contaminants. If these procedures are not properly controlled they may adversely affect the viability of tubercle bacilli, resulting in false-negative cultures. In general, if less than 2% of a laboratory's mycobacterial cultures become overgrown with bacterial or fungal contaminants, the decontamination procedure is overly harsh and may be inhibiting/preventing growth of tubercle bacilli. On the other hand, false-negative cultures may also result when inadequate decontamination procedures allow overgrowth of the medium by contaminating organisms.

False negative cultures may also occur if there are inordinate delays between specimen collection and processing that allow progressive dying-off of tubercle bacilli. Lastly, false-negative cultures may result when incubation is not done for a full eight weeks, since some tubercle bacilli (particularly some drug-resistant strains) may require extended periods of incubation to produce visible growth.

Culture is much more costly than microscopy, requiring facilities for media preparation as well as skilled staff. Culture should be used selectively, in the following order of priority:

Box 1.1 Selective use of culture

- Surveillance of tuberculosis drug resistance as an integral part of the evaluation of control programme performance
- Diagnosis of cases with clinical and radiological signs of pulmonary tuberculosis where smears are repeatedly negative
- Diagnosis of extra-pulmonary and childhood tuberculosis
- Follow-up of tuberculosis cases who fail a standardised course of treatment and who may be at risk of harbouring drug resistant organisms
- Investigation of high-risk individuals who are symptomatic, eg. laboratory workers, health care workers looking after multidrug resistant patients

1.3 Drug susceptibility testing

Drug susceptibility testing of *M. tuberculosis* isolates is of considerable value for epidemiological purposes. The prevalence of resistance in new tuberculosis patients (primary resistance) is a good measure of the efficiency of treatment services and guides the choice of regimens in treatment programmes. Trends in resistance among previously-treated patients (acquired resistance) point to failures in control programme management. Susceptibility tests may also be of value for individual patients if there is a failure during chemotherapy or a relapse after successful treatment. However, routine susceptibility testing of cultures from new patients imposes an unrealistic burden on laboratory services and with the documented success of four-drug treatment regimens (even in the presence of single-drug resistance), its benefit cannot be justified.

Box 1.2 Drug susceptibility is mainly of value for epidemiological purposes. Testing of individual patients should be limited to:

- Patients who fail standardised treatment regimens
- High risk individuals who are found to have positive cultures, eg. laboratory workers, health care workers looking after multidrug resistant patients
- Close contacts of multidrug resistant tuberculosis patients who have signs and symptoms of tuberculosis

1.4 Species identification

Identification of mycobacterial species may have value in countries where tuberculosis incidence is low and where a substantial number of mycobacterial isolates may be of other species, particularly in HIV-infected patients. However, in developing countries where more than 85% of the disease burden is due to tubercle bacilli, species identification other than *M. tuberculosis* is of little value.

2 ORGANIZATION OF LABORATORY SERVICES

2.1 Integrated versus specialised services

Before the introduction of effective anti-tuberculosis drugs, the bacteriology of tuberculosis was usually confined to examination of smears at bacteriology departments of general hospitals or at tuberculosis dispensaries and clinics. Culture, guinea pig inoculations and identification of tubercle bacilli were almost exclusively done in laboratories of specialised sanatoria or tuberculosis hospitals.

Following the introduction of anti-tuberculosis chemotherapeutic agents after World War II, patients rapidly became non-infectious and were no longer isolated in sanatoria for long periods of time. Tuberculosis patients were treated in general hospitals as out-patients and tuberculosis bacteriology moved away from specialised laboratories into those of more general pathology departments. Unfortunately, this resulted in sub-optimal methods in some laboratories while others were hampered by a lack of experience and interest. Today it is still not unusual for health care workers to comment on the variation in the quality of technical assistance which they receive from laboratories, and strong arguments sometimes develop for tuberculosis bacteriology to once again become the domain of specialised laboratories.

The obvious advantage of exclusive tuberculosis laboratory services lies in dedication to tuberculosis bacteriology (often lacking in integrated services). Any technique will give better results when it is applied by specially trained workers as their only activity, than by persons who apply it occasionally and as one among many activities. However, the only way in which tuberculosis control can be applied on a community-wide scale in any country is through the general health service and within the framework of primary health care. When a technique (such as microscopy) has to be applied everywhere and over a long period - often permanently - the operational aspects must take precedence over the technological. Peripheral laboratories (and in some countries even regional laboratories) for tuberculosis should, therefore, be integrated within the public health laboratory system. The first aim should be to achieve “quantity”, ie. a complete extension of peripheral laboratory services and full coverage, and then to follow this closely by achieving “quality” through continuous training and supervision.

However,

Extension of tuberculosis laboratory services should not outpace the extension of DOTS coverage in countries

Some of the laboratory techniques used in tuberculosis bacteriology do require complicated and expensive technology as well as equipment that is difficult to maintain. Furthermore, laboratory workers have a well-defined risk of tuberculosis

infection if proper precautions are not taken. These arguments favour the establishment of specialised tuberculosis laboratory services at the higher levels of the health service.

2.2 Levels of laboratory services

Tuberculosis laboratory services should form part of integrated tuberculosis control programmes, which in turn should form part of overall primary health care programmes of countries. It follows, therefore, that tuberculosis laboratory services should be organised according to the three levels of general health services, ie:

- the peripheral (often district) laboratory
- the intermediate (often regional) laboratory
- the central (often national) laboratory

In terms of technical complexity, the activities performed at each level are different:

Peripheral laboratories should be capable of performing sputum smear microscopy utilising Ziehl-Neelsen (ZN) staining of unconcentrated sputum specimens from tuberculosis suspects. Peripheral laboratories should be fully integrated with primary health care services and could be based at primary health care centres or district hospitals.

Intermediate laboratories should be capable of providing supervision, monitoring, training and quality assurance to peripheral laboratories. Fluorochrome staining of sterilised concentrated specimens in addition to ZN procedures may be done, if dictated as necessary by the load of specimens. Mycobacterial culture of clinical specimens and differentiation between *M. tuberculosis* and other mycobacterial species could be performed in regional laboratories. These could be integrated with existing public health laboratories in bigger hospitals or in cities, provided that dedicated tuberculosis bacteriology sections can be identified.

Central laboratories should be at the apex of health laboratory structures and should be capable of performing microscopy (both ZN and fluorescence), mycobacterial culture, drug susceptibility testing and species identification. These laboratories may be separate from public health laboratories and could reside in research institutions or in a country's principal tuberculosis or public health institution. Aside from the technical activities pertaining to these reference centres, national laboratories should provide training for laboratory staff, perform quality assurance and proficiency testing, exercise surveillance of primary and acquired tuberculosis drug resistance and participate in epidemiological and operational research.

In the early phases of development of a laboratory service for tuberculosis in a high prevalence country the most economical and efficient arrangement is as follows:

1. Establishment of ZN microscopy in small, multi-purpose public health laboratories. Caution is, however, necessary when establishing peripheral microscopy sites, since a direct relationship exists between workload, number of microscopists required and the quality of microscopy performed in these small laboratories. The maximum number of ZN smears examined per microscopist per day should not exceed 20. If more examinations are attempted, visual fatigue will lead to a deterioration of reading quality. On the other hand, proficiency in reading ZN smears can only be maintained by examining at least 10 to 15 ZN smears per week, ie. a minimum of 2-3 examinations per day.

   One microscopy centre per 100 000 population is usually sufficient to attain the target of 2-20 ZN smears per day.

   In densely-populated areas fewer laboratories would be required if transport and communication mechanisms could be improved, while in remote and sparsely populated areas more laboratories may be needed. Therefore, in planning microscopy services, careful consideration should be given to the following aspects:

   - location and utilisation of existing services (if any)
   - population distribution
   - transport facilities
   - expected workload based on the recommendations for case detection, diagnosis and monitoring of treatment

   Annex 1 provides an example of how to assess whether the number of microscopy centres in a country is adequate.

2. Establishment of fluorescence microscopy at regional laboratories where more than 100 smears are examined per day. Since low magnification is used, screening of a smear can be up to five times faster. Fluorescence microscopy requires much more expertise and experience and the capital cost and running expenditure are considerable. Also, it is necessary to retain Ziehl-Neelsen microscopy to confirm positive smears found by fluorescence microscopy, especially if microscopists are inexperienced with regard to fluorescence microscopy, and for training and quality assurance of the peripheral laboratories..

   One fluorescence microscopy centre per 500 000 to one million population is usually sufficient. However, this is much more strongly dictated by the daily case load than by the actual population covered.

3. Establishment of tuberculosis culture facilities at regional or central level, to cover 500 000 to one million population. Specimens from peripheral health centres should reach the culture laboratory within five days. Since the capital cost of equipment and its satisfactory maintenance are much larger items of expenditure than staff salaries, it is usually not cost-effective to use highly

simplified culture procedures which are less efficient than slightly more complicated methods. For example, culture methods employing a centrifuge are more efficient than simple decontamination and culture of sputum directly onto medium. The additional cost of a centrifuge and the time taken in processing the specimen is very small compared to the total running cost of the laboratory.

4. Establishment of a central reference laboratory at national or regional level, to cover 10 million or more population. In small countries one central reference laboratory should be established, even if the population is below 5 million. In large countries, several such laboratories may be established, but one of these should be designated the national reference laboratory.

As bacteriological services extend and as health care workers begin to utilise bacteriological methods in preference to radiography, there will be an increasing demand for smear and culture examinations. The most economical way is then to establish fluorochrome microscopy in busier microscopy centres and to increase the number of culture facilities.

2.3 Functions and responsibilities of peripheral, intermediate and central laboratories

Tuberculosis laboratory services cover various activities, which differ from country to country and even from region to region within a country. These can be summarised as follows:

- detection of acid-fast bacilli by microscopy
- bacteriological culture of clinical specimens for mycobacteria
- identification of mycobacterial species
- performance of drug susceptibility tests
- performance of quality assurance and proficiency testing
- consultation with health care workers on the diagnosis and management of tuberculosis
- collection and analysis of laboratory data for epidemiological purposes
- teaching and training of laboratory staff
- participation in epidemiological and operational research

Obviously, not all of these activities can or should be carried out by every laboratory. The functions and responsibilities of the various levels of laboratory services can be summarised as follows:

PERIPHERAL LEVEL

Technical
- preparation and staining of smears
- ZN microscopy and recording of results
- internal quality control

Administrative
- receipt of specimens and dispatch of results
- cleaning and maintenance of equipment
- maintenance of laboratory register
- management of reagents and laboratory supplies

INTERMEDIATE LEVEL

All the functions of the peripheral level, plus:

Technical
- fluorescence microscopy (optional)
- digestion and decontamination of specimens
- culture and identification of *M. tuberculosis*
- preparation and distribution of reagents for microscopy in peripheral laboratories

Managerial
- training of microscopists
- support to and supervision of peripheral staff with respect to microscopy
- quality improvement and proficiency testing of microscopy at peripheral laboratories (section 7, page 41)

CENTRAL LEVEL

All the functions of the intermediate level, plus:

Technical
- drug susceptibility testing of *M. tuberculosis* isolates
- identification of mycobacteria other than *M. tuberculosis*

Administrative
- technical control of and repair services for laboratory equipment
- updating and dissemination of manuals on bacteriological methods for diagnosing tuberculosis

- development and dissemination of guidelines on care and maintenance of microscopes and other equipment used in tuberculosis bacteriology
- development and dissemination of guidelines on tuberculosis laboratory supervision and quality assurance
- collaboration with the central level of the National Tuberculosis Programme in defining technical specifications for equipment, reagents and other materials used in bacteriological investigations, and in estimating laboratory materials and equipment requirements for the Programme budget

Managerial

- training of intermediate laboratory staff in bacteriological techniques and support activities, ie. training, supervision, quality assurance, safety measures and equipment maintenance
- supervision of intermediate laboratories regarding bacteriological methods and their support (particularly training and supervision) to the peripheral laboratories
- quality assurance of microscopy and culture performed at intermediate laboratories (section 7, page 41)

Research and surveillance

- organization of surveillance of primary and acquired mycobacterial drug resistance
- operational and applied research relating to the laboratory network, co-ordinated with the requirements and needs of National Tuberculosis Programmes.

3 TRAINING, SUPERVISION AND MOTIVATION OF LABORATORY STAFF

If a tuberculosis laboratory is to function effectively, motivated and dedicated staff are crucial. *Laboratory personnel must be fully aware of their important role in tuberculosis control and must become full partners in National Tuberculosis Programmes.* Training laboratory technicians in the microscopic diagnosis of tuberculosis is, accordingly, an essential activity under the revised tuberculosis control strategy.

One of the biggest problems that arise in laboratories in developing countries concerns the supply, maintenance and repair of equipment, the supply of laboratory consumables and transport. Solutions to these problems require fairly intensive technical training, a knowledge of laboratory administration and management and the development of interpersonal skills. It takes much longer - and is at least as important - to teach peripheral microscopists how the use their microscopes properly, (including maintenance and repair), and how to conduct the day-to-day running of a microscopy laboratory, (including planning of activities and time scheduling) than it is to teach them how to prepare and read slides.

3.1 Training

3.1.1 *Peripheral laboratory staff*

Technicians working in peripheral microscopy laboratories must receive training on the following:

- the relevance of sputum-smear microscopy to tuberculosis diagnosis, follow-up during treatment and treatment evaluation
- the importance of carrying out all requested sputum-smear examinations
- performing Ziehl-Neelsen staining
- timely reading and reporting of the results in a timely fashion

Laboratory technicians should have elementary knowledge of mathematics and the metric system, use of laboratory equipment and instruments, and measures for ensuring the safety of laboratory personnel and laboratory premises. They should also have an understanding of the concepts of asepsis and sterilisation.

Training in microscopy should specifically cover:

- collection, storage and transport of sputum specimens for microscopy
- smear preparation, including numbering / engraving of slides, selection of useful particles, fixation, staining, decolourisation and counter staining
- use of a microscope with an immersion-oil objective and slide reading
- reporting of results and recording of data in the Tuberculosis Laboratory Register

- procedures for reporting results to the peripheral health structure and / or the patient
- maintenance and minor repairs of microscopes
- storage of positive slides and negative slides for quality assurance
- procedures for sending sputum specimens for culture and drug susceptibility testing
- disinfection and sterilisation of contaminated material
- safety measures for handling sputum specimens and performing microscopy
- identification of problems occurring during sputum-smear microscopy and recording of results
- management of reagents and laboratory supplies

Intermediate laboratories are responsible for organising and conducting the training for the peripheral laboratory staff from each district.

Training should be essentially practical and held over five days. The number of technicians to be trained simultaneously will depend on the available materials and equipment, especially microscopes, to be used for training purposes. On average, one microscope is required for every two technicians to be trained. A separate room for training should be arranged at the intermediate laboratories, for a maximum of 10 trainees per course.

3.1.2 *Intermediate laboratory staff*

Laboratory staff at intermediate laboratories should be trained in the technical methods required and the managerial functions they must undertake within the National Tuberculosis Programme.

Training should cover:

- the DOTS strategy, including general information on the National Tuberculosis Programme and the functions of the tuberculosis laboratory network
- technical methods:
  - Ziehl-Neelsen microscopy (as in the curriculum for peripheral laboratory staff)
  - preparation of reagents for Ziehl-Neelsen microscopy
  - fluorescence microscopy, if equipment is available
  - Löwenstein-Jensen culture procedures, including preparation of sputum specimens for culture, inoculation of media, media incubation, reading, recording and reporting of results

- managerial skills:
  - organization of training on Ziehl-Neelsen microscopy for peripheral laboratory staff
  - supervision of peripheral laboratory staff
  - quality control of microscopy at peripheral laboratories
  - organization of transport of sputum specimens within districts and from districts to the intermediate laboratory
  - estimating supply and equipment requirements for programme budgeting

The national reference laboratory, in collaboration with the other Central laboratories, is responsible for organising the training of intermediate laboratory personnel. The training should take place over two or three weeks.

3.1.3 Central laboratory staff

Central level staff must in addition be trained in drug susceptibility testing techniques and surveillance methods, identification of mycobacterial species, evaluation of laboratory activities and operational research methodology. They can be trained within the country, or can attend international training courses sponsored by WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD).

3.2 Supervision

The regional Laboratory Supervisor is responsible for monitoring the day-to-day activities of the peripheral laboratories, and for training and updating staff on all aspects of sputum smear microscopy. The Supervisor must also ensure that laboratory activities are carried out as planned and should perform quality control and proficiency testing. The Supervisor should visit the peripheral laboratories once every four to eight weeks and should work with the District Tuberculosis Co-ordinator to make sure that tuberculosis-related laboratory activities are performed properly.

Supervisory visits should be planned carefully and the laboratory supervisor should keep a checklist of the items to be checked during supervisory visits. Items for checking are usually divided into four categories:

- *Competence of the laboratory technician*

  The Supervisor should ensure that the laboratory technician knows:

  - how to prepare sputum-smear slides for Ziehl-Neelsen microscopy
  - how to read slides and record results
  - how to complete the Tuberculosis Laboratory Register accurately and how to report the results
  - how information from the Tuberculosis Laboratory Register can be used to cross-check information in the District Tuberculosis Register
  - how to limit administrative errors in the identification of patients
  - how to estimate laboratory supplies and reagents

- *Activities of the laboratory technician*

  The Supervisor should ensure that the laboratory technician:

  - assesses sputum-smear microscopy through quality control
  - performs examination of sputum specimens for all respiratory symptomatics: three sputum specimen slides if they are negative, and at least two sputum specimen slides if they are positive
  - keeps the Tuberculosis Register up to date and completes it accurately
  - keeps a box of all smear-positive slides and another of selected smear-negative slides for quality control

- *Consistency of the Laboratory and District Registers*

  The Supervisor should ensure that:

  - the smear-positive patients registered in the Tuberculosis Laboratory Register are also registered in the District Tuberculosis Register
  - the smear results for follow-up patients in the Tuberculosis Laboratory Register are the same as those recorded in the District Tuberculosis Register

- *Logistics*

  The Supervisor should ensure that:

  - the supply of sputum containers, slides, reagents, forms and other laboratory materials is adequate
  - that the binocular microscope is in good working order

3.3 Motivation

Motivation of staff is a neglected issue in tuberculosis bacteriology. It has to be realised that most people dislike manipulating sputum and that microscopy of largely negative smears can become very boring. Moreover, staff in peripheral laboratories often feel isolated and neglected; feelings of frustration are regularly expressed because they often find themselves at the receiving end of blame without being involved in National Tuberculosis Control Programme activities - “the last to be informed but the first to be blamed”.

Motivation may be fostered in several ways:

- associating laboratory staff with the clinical and epidemiological aspects of tuberculosis control by arranging visits to clinics and hospitals, and talks and demonstrations by health care workers. This will help laboratory staff to appreciate the problems of tuberculosis at the patient level and enable them to identify with a team

- involving laboratory staff in planning and decision making processes through active participation in meetings and discussions with other members of the tuberculosis control team

- giving priority to visiting the laboratory during control programme supervisory visits; by using the laboratory register as the initial tool to evaluate case detection and quality of registration and follow-up of cases, and by discussing the functioning of the control programme jointly with nursing, clinical and laboratory staff
- organising inter-laboratory visits and meetings to discuss mutual problems. This will alleviate the sense of isolation and could lead to innovative solutions to problems that may be perceived as overwhelming
- including laboratory staff in regular feedback sessions on the outcome and performance of tuberculosis control programmes. Working in a successful programme may become a good motivational factor
- providing regular refresher courses on the different aspects of tuberculosis microscopy and awarding staff who complete these courses with certificates

Job satisfaction is a well-known principle in management: a person will perform a job well only if s/he is interested in it and finds it psychologically - if not financially - rewarding. There is no easy answer to the problem of staff motivation - or lack of it - in tuberculosis bacteriology. Nevertheless, aside from training and supervision, support and motivation of laboratory staff should also become an essential activity under the revised tuberculosis control strategy.

4 LABORATORY ADMINISTRATION AND RECORD KEEPING

The purpose of a laboratory recording and reporting system is to provide information to improve the management of the National Tuberculosis Programme at all levels (national, regional and district).

Accurate record-keeping of specimens received, specimen processed, laboratory results and specimens sent to referral or regional laboratories for culture and susceptibility testing is essential for the proper management of the control programme strategy.

Standardised records should be simple, practical and limited to essential information. Laboratory Supervisors should assess the quality, review the specimen request forms, laboratory register, and reporting of the laboratory results for completeness, consistency and credibility.

4.1 Standard operating procedures

Written operating and cleaning instructions must be kept in a file for all equipment. Dated service records must be kept for all equipment. Laboratory procedures used routinely should be those that have been published in reputable microbiological books, manuals or journals. Every procedure performed in the laboratory must be written out exactly as carried out and be kept in the laboratory for easy reference. Any changes must be dated and initiated by the Laboratory Supervisor.

All laboratory records should be retained for two years.

4.2 Laboratory request forms

This form is sent with the specimen (or patient), requesting the appropriate examination. The patient's data must be completed in full and the examination requested should be noted eg. sputum microscopy, culture, etc.

Model laboratory request forms are presented in Annex 2.

4.3 Laboratory registers

This is a record book maintained by the technician/technologist in the laboratory responsible for sputum smear examination and/or sputum culture of tuberculosis suspects and follow-up examinations. For each tuberculosis suspect, the Tuberculosis Laboratory Register should contain the following:

- Date of specimen received
- Laboratory reference number
- Type of specimen received
- Patient name, gender, age

- Patient register number (if available)
- Smear and/or culture results
- Results of confirmatory tests for *M. tuberculosis* (if applicable)
- Date results reported
- Name of person responsible for tests

Model laboratory registers are presented in Annex 3.

4.4 Laboratory report forms

These forms contain the results of microscopic and/or culture examination and should clearly state the outcomes as described in the Technical Series on Microscopy and the Technical Series on Culture. It is also important to indicate whether results are preliminary (eg. awaiting culture) or final.

Model laboratory report forms are presented in Annex 4.

4.5 Laboratory accident book

This book should be kept by the Laboratory Supervisor and should contain extensive details about laboratory accidents and the necessary measures taken. Each laboratory accident should be reported to the person in charge and full details entered into the laboratory accident book, noting the following:

- Date of the accident
- Name of person concerned
- Description of accident
- Laboratory number of specimen/strain involved
- Extent of injury
- Containment and follow-up measures taken

Both the laboratory supervisor and the person who caused the accident should sign the statement.

LABORATORY HYGIENE AND SAFETY

With the escalation of tuberculosis world-wide, driven by the HIV epidemic and aggravated by the emergence of multidrug-resistance, renewed concern has arisen about safety in tuberculosis laboratories.
Studies have shown that the risk of tuberculosis infection is three to five times higher for laboratory workers when compared to administrative staff or the general community, depending on the type of laboratory work done. The potentially dire consequences of becoming infected with multidrug-resistant strains of *M. tuberculosis* and the increasing proportion of persons infected with HIV (which include laboratory workers) add a new dimension to the problem and emphasise the importance of strict adherence to safety precautions in the laboratory.

M. tuberculosis is included in Risk Group III in the 1983 WHO classification of risk, along with other micro-organisms most likely to infect laboratory workers by the airborne route. The number of tubercle bacilli required to initiate infection is low, the infective dose being less than 10 bacilli. Infective particles in the laboratory are usually derived from moist droplets discharged into the air by procedures liberating aerosols. When aerosolised material dry out droplet nuclei of 1µm to 5µm in size are formed creating infective particles which may remain in the air for long periods of time.

Safety precautions in tuberculosis laboratories must involve measures to:

- minimise the production and dispersal of aerosols and infective particles
- prevent laboratory workers from inhaling airborne particles and
- prevent infection by accidental inoculation and ingestion

The focus of bio-safety in the tuberculosis laboratory should be on primary containment measures which are aimed at protecting laboratory staff and the immediate environment. Appropriate ventilation should flow from clean to contaminated areas. In peripheral laboratories in tropical countries windows may be necessary; however, these should be located in such a way that air currents do not pass over the area of smear preparation in the direction of the laboratory worker preparing the smears. In culture laboratories air should be continuously extracted to the outside of the laboratory at a rate of six to twelve air changes per hour. Supply and exhaust air devices should be located on opposite walls, with supply air provided from clean areas and exhaust air taken from less clean areas. Exhaust air must be discharged directly to the outside of the building to be dispersed away from air intakes.

Patients with tuberculosis are increasingly co-infected with HIV and their specimens may either be contaminated with blood or be a primary source of the virus, eg. blood or bone marrow specimens. *Attention should also be focused on the increased risk of laboratory workers contracting tuberculosis should they be or become infected with HIV.*

Safety in the tuberculosis laboratory must start at the administrative level. It is an administrative responsibility to ensure that laboratory staff are:

- trained properly in safe laboratory procedures
- informed of *especially dangerous techniques* and procedures that require special care (see 5.1 below)
- provided with adequate safety equipment and clothing
- prepared for prompt corrective action following a laboratory accident
- educated about their increased risk of acquiring tuberculosis should they be or become HIV positive
- monitored regularly by medical personnel

The laboratory worker is responsible for:

- following established laboratory policy
- accepting the responsibility for correct work performance to assure the safety of fellow workers
- using appropriate safety equipment
- accepting the responsibility for maintaining a health lifestyle

The most expensive and sophisticated equipment is no substitute for safe techniques and meticulous care. Good hygiene practices and adherence to safety procedures are the responsibility of every laboratory worker

5.1 Procedural hazards

5.1.1 Inhalation hazards

Most infections in the tuberculosis laboratory can be attributed to the unrecognised production of potentially infectious aerosols containing tubercle bacilli. Many microbiological techniques generate aerosols, eg. when bubbles burst or when liquids are squinted through small openings or impinge on surfaces. Large (>5μm) aerosolised droplets settle rapidly to contaminate skin, clothing and counter tops; however, the most dangerous aerosols are those that produce droplet nuclei - tiny dry particles less than 5μm in size that may contain one or more viable tubercle bacilli. Droplet nuclei can float in the air almost indefinitely and are inhaled and trapped in the lung alveoli where they initiate infection.

The following microbiological activities generate aerosols:

- *Collecting sputum specimens from coughing patients*

  Tuberculosis suspects are sometimes referred directly to the laboratory for sputum collection. This practice exposes laboratory workers to a high risk of infection by aerosols produced during collection procedures. Precautions to lower this risk include instructing tuberculosis suspects to cover their mouths while coughing, standing behind (and not in front of) coughing individuals and collecting specimens outdoors where aerosols are diluted and sterilised by direct sunlight.

- *Adding decontamination solutions*

  Decontamination solutions should be added gently and never be mixed while containers or tubes are open. After the digestion / decontamination procedure, pour the supernatant fluid through a funnel into a splash-proof container to minimise both aerosol production and contamination of the work surface. Gently discard the fluid down the side of the funnel into an appropriate disinfectant solution (eg. 5% phenol).

- *Working with bacteriological loops*

  Loops should be handled gently and vigorous spreading of inocula on medium or from cultures should be avoided. Loops containing infectious material should first be cleaned by rotating them in a 250ml screwcap flask containing 70% alcohol and washed sand. They can then be sterilised in a Bunsen flame, the alcohol causing rapid incineration of residual debris.

- *Pipetting*

  Never pipette by mouth. Not only is there a danger of aspirating infectious material, but aerosols are created when fluid is alternately sucked and expelled through the pipette. With the nose directly over the open container of infectious material, the aerosols have direct access to the lungs.

  Pipettes should be drained gently and not blown out violently, otherwise the last drop will form bubbles which burst and create aerosols. Pasteur pipettes are particularly likely to generate bubbles.

- *Centrifugation*

  The recommended type of centrifuge for tuberculosis laboratories is a floor model with a lid and fixed angle rotor which contains sealed centrifuge buckets or aerosol-free carriers. Centrifuges should preferably be fitted with an electrically operated safety catch which prevents the lid from being opened while the rotor is spinning.

  Tubes should always be capped during centrifugation and balanced within centrifuge buckets to avoid breakage. If centrifuge tubes leak, crack or shatter during centrifugation, an invisible cloud of potentially infectious droplet nuclei may be released.

- *Pouring into disinfectant*

  When infectious material is poured into disinfectant it may splash and create aerosols, while contaminating surrounding surfaces. Use a funnel with its end beneath the surface of the disinfectant and gently discard the material down the side of the funnel.

5.1.2 *Ingestion hazards*

Tuberculosis materials may be ingested by direct aspiration through mouth pipetting and by putting into the mouth fingers and objects that have been contaminated while in contact with the laboratory bench. Fingers may also become contaminated by the outside of specimen containers. Routine disinfection of containers before processing is recommended and frequent hand washing should be routine practice.

5.1.3 *Inoculation hazards*

Needle stick accidents are not uncommon and hypodermic needles should never be used as a substitute for pipettes. Cuts from contaminated glassware may also occur and touching of broken glassware must be avoided. Glass Pasteur pipettes are the most dangerous and glassware should be substituted with plastic materials whenever possible.

5.2 Laboratory hygiene

- Entry to the laboratory should be restricted to laboratory staff only
- Eating, drinking, smoking or applying make-up must be prohibited in the laboratory
- Mouth-pipetting, licking labels and sucking pencils should not be allowed
- Hands must be washed with a suitable bactericidal soap upon entering the laboratory, after handling potentially contaminated specimen containers, after any bacteriological procedure, after removing protective clothing and before leaving the laboratory. Disposable paper towels should be used for hand drying
- No cleaning, service or checking of equipment should be allowed unless a trained technical or professional person is present to ensure adequate safety precautions
- All surfaces and equipment within the laboratory should be regarded as potentially infectious and should be cleaned regularly by appropriate means. Floors should not be waxed or swept but should be mopped regularly to limit dust formation

5.3 Disinfectants

The temporal killing action of disinfectants depends on the population of organisms to be killed, the concentration used, the duration of contact and the presence of organic debris.

The proprietary disinfectants suitable for use in tuberculosis laboratories are those containing phenols, hypochlorites, alcohols, formaldehydes, iodophors or glutaraldehyde. These are usually selected according to the material to be disinfected. Sweet-smelling "anti-septics" should not be used. It is incorrect to assume that a disinfectant which has general usefulness against other micro-organisms is effective against tubercle bacilli. A number of commercially available disinfectants have no or little mycobactericidal activity, while quaternary ammonium compounds are not effective at the recommended concentrations.

Disinfectant solutions should be prepared fresh each day and should not be stored in diluted form because their activity will diminish.

Phenol should be used at a concentration of 2% to 5% and contact time should be 15-30 minutes, depending on the type and volume of material to be disinfected. Phenol is useful in soaked paper towels to cover working surfaces. This minimises spatter and aerosol formation in the event of spilling.

Hypochlorite should be used at concentrations between 1% and 5%, with a contact time of 15-30 minutes, depending on the type and volume of material to be disinfected. Hypochlorite solutions (5%) are useful for the disinfection of material containing organic debris because of their digesting action.

Glutharaldehyde does not require dilution but an activator (provided separately by the manufacturer) must be added. Glutaraldehyde is usually supplied as a 2% solution, while the activator is a bicarbonate compound. Glutaraldehyde is useful for decontaminating bench surfaces and glassware. The activated solution should be used within two weeks and discarded if turbid.

Alcohols, usually 70% ethanol (methylated spirits) or propanol is used in alcohol-sands baths and for decontaminating benches and surfaces. It should also be used instead of water to balance centrifuge tubes. When hands become contaminated, a rinse with 70% isopropyl alcohol followed by thorough washing with soap and water is effective.

Iodophor preparations should be used at concentrations of 3% to 5% and contact time should be 15-30 minutes, depending on the type and volume of material to be disinfected. Iodophors are useful for mopping up spills and for handwashing.

All of the above disinfectants are toxic and undue exposure may result in respiratory distress, skin rashes or conjunctivitis. However, used normally and according to the manufacturers' instructions, they are safe and effective.

5.4 Essential safety equipment and supplies

5.4.1 *Biological safety cabinets*

The single most important piece of laboratory safety equipment needed in the tuberculosis culture laboratory is a well-maintained, properly functioning biological safety cabinet (BSC).

BSCs use high efficiency particulate air (HEPA) filters in their exhaust and/or air supply systems. HEPA filters remove particles ≥ 3 µm (which essentially includes all bacteria, spores and viruses) with an efficiency of 99.97%.

Two types of cabinets can be used in tuberculosis culture laboratories: One is a Class I negative-pressure BSC that draws a minimum of 75 linear feet of air per minute (22.86 meter per second) across the front opening and exhausts 100% of air to the outside. Class I BSCs provides protection to the user (ie. laboratory worker) but not to the product (ie. cultures for tubercle bacilli). Strict precautions are, therefore necessary to avoid contamination of culture media. The other BSC is a Class II vertical laminar flow cabinet that blows HEPA filtered air over the work area. Because cabinet air has passed through the HEPA filter, it is contaminant free and may be circulated back into the laboratory (Type A) or ducted out of the building (Type B).
The airflow through the BSC is adjusted by the manufacturer to provide at least 75 linear feet per minute (22.86 meter per second) and should be tested and re-certified once a year by trained personnel. Regular checks (eg. quarterly, or more often under dusty conditions) on the airflow should be made with an anemometer. If the airflow is appreciably diminished this indicate that the filters have become clogged and that the BSC needs to be decontaminated.

The following safety precautions need to be observed when working within a BSC:

- Use proper microbiological techniques to avoid splatter and aerosols. This will minimise the potential for staff exposure to infectious materials manipulated within the cabinet. As a general rule, keeping clean materials at least 12cm away from aerosol-generating activities will minimise the potential for cross-contamination
- Do not hold opened tubes or bottles in a vertical position and recap or cover them as soon as possible. This will reduce the chance for cross-contamination
- Do not use open flames in the BSC. This creates turbulence which disrupts the pattern of air supplied to the work surface. Small electric furnaces are available for decontaminating loops and are preferable to an open flame inside the BSC
- Use an appropriate liquid disinfectant in a discard pan to decontaminate materials before removal from the BSC. Introduce items into the pan with the minimal splatter and allow sufficient contact time before removal. Alternatively, contaminated items may be placed into an autoclavable disposal bag within the BSC. Water should be added to the bag prior to autoclaving to ensure steam generation during the autoclave cycle

BSCs have to be decontaminated at least once a year and the filters replaced by creating a formaldehyde vapour which will kill all tubercle bacilli trapped in the filters. Always decontaminate the BSC before HEPA filters are changed or internal repair work is done. The most common decontamination method uses formaldehyde gas created by adding potassium permanganate to formaldehyde liquid, as indicated by Diagram 1 on page 33.

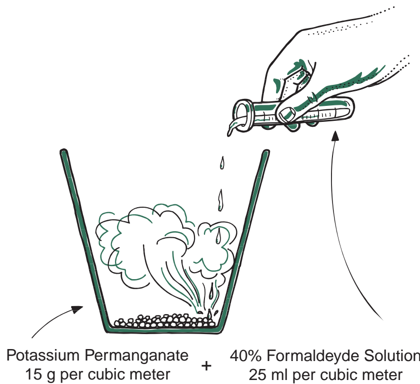
Diagram 1

Decontamination of BSC before removing filters and after laboratory accidents.

PROCEDURE

Remove all material and equipment from the BSC and from the immediate environment. Ensure that the BSC is switched on

Seal all air intake and exhaust grills in the laboratory by taping large plastic garbage bags over the grills. Also tape around door frames or other openings through which the formaldehyde vapour may leak



Use 25ml of a 40% formaldehyde solution and 15g potassium permanganate for each cubic meter capacity that has to be decontaminated:

Place the potassium permanganate crystals in a deep metal container in the BSC. Pour the formaldehyde solution over the crystals and *leave the laboratory immediately* since the reaction rapidly produces the release of heat and formaldehyde gas. Close and seal the laboratory door

Allow the formaldehyde vapour to act overnight (and preferably over a weekend), with the BSC being switched on

Remove the covers from air intake and exhaust grills as well as the tape around doors and other openings. Allow the room to air until no more formaldehyde is detectable, then mop all residue from the floors, walls and benches. If a white, powdery residue is obvious, remove by wiping with a 10% ammonium hydroxide solution (use gloves)

Switch the BSC off and proceed with replacement of filters or repair

5.4.2 Protective clothing

Suitable clothing must be worn while working in the laboratory:

- *Coats* and *gowns* should wrap across the body and cover the upper chest and neck. Sleeves should be wrist length
- *Gloves* should be worn when handling potentially infectious materials or when touching potentially infectious surfaces or equipment. Gloves also guard against infection through cuts or abrasions on the hands. Disposable gloves should not be re-used
- Industrial *face masks* designed to filter >95% of particles ranging from 1-5µm could be worn during aerosol-producing procedures, especially in culture laboratories. Masks should be discarded after eight hours of use

Protective clothing should be removed before leaving the laboratory and should be placed in covered containers or laundry bags before being washed or discarded.

5.5 Coping with a laboratory accident

Laboratory safety does not just happen. It is the result of:

- recognising that accidents can and will occur
- formulating a plan of action to neutralise the potential harmful effects of an accident as rapidly and effectively as possible
- discussing ways to minimise and prevent accidents from occurring

The best defence against a laboratory accident is a well-thought-out plan to neutralise its effects as quickly and effectively as possible. *No accident should be considered insignificant*; however, assessment of the seriousness of each accident is necessary to determine the most appropriate course of action.

- Be prepared for an accident by having the following readily accessible in or near areas where accidents are most likely to occur:
  - a supply of paper towels or large cloths
  - a wide-mouthed (to facilitate rapid pouring) container of disinfectant
  - a supply of industrial face masks capable of filtering particle sizes between 1µm and 5µm

A fogging machine is useful to quickly disperse disinfectant into a room in the case of an accident. The mist released by the machine rapidly saturates the air, causing the dangerous droplet nuclei to settle. An appropriate disinfectant used in the fogging machine will decontaminate potentially infectious droplet nuclei as they settle on floors and bench tops. The fogging machine should always be kept ready with an adequate volume of freshly-prepared disinfectant.

Accidents that occur in tuberculosis laboratories may be divided into two types: those that generate limited aerosols and those that produce a large volume of potentially infected aerosols. A limited aerosol may, for example, be created by breaking a single culture tube of egg medium or spilling the contents of a sputum specimen. In these instances, the solid medium and thick mucoid nature of the sputum specimen greatly limit large numbers of tubercle bacilli from being aerosolised. A plan of action for limited aerosol accident is presented in Diagram 2 on page 35.

A *large volume of potentially infectious aerosols* may be generated by breaking one or more tubes of liquid containing a high concentration of tubercle bacilli, eg. bacterial suspensions or unbalanced centrifuge tubes. A plan of action for a large volume aerosol accident is presented in Diagram 3 on page 36.

5.5.1 Accidents within the BSC

In the event of any spillage within the BSC, *the cabinet should not be switched off*. A plan of action for a limited aerosol accident in the BSC is presented in Diagram 4 on page 37. A plan of action for a large volume aerosol accident in the BSC is presented in Diagram 5 on page 38.

Diagram 2

Plan of action for limited aerosol accident

PROCEDURE

Cover the spill immediately to prevent further aerosolisation. Use any available material, eg. paper towels, newspapers or even a laboratory coat

Soak the cover with appropriate disinfectant and completely wet the area

Let stand for at least two hours, keeping the area wet during this time

Place all broken tubes/containers and clean-up material in an appropriate container and discard by one of the waste disposal options described later

Mop the floor and laboratory benches with appropriate disinfectant

Diagram 3

Plan of action for large volume aerosol accident

PROCEDURE

Evacuate the room immediately, except for the person who caused the accident

Shut off the air system (if appropriate). Seal the exhaust and intake air ducts as quickly as possible (use plastic garbage bags and seal with tape)

Turn the fogging machine on, exit the room and seal the door with tape

Let the fogging machine dispense the entire volume of disinfectant, allow the fog to settle and leave the room undisturbed for at least two hours

Put on protective clothing (including an industrial mask) before re-entering the room

Soak the spill with appropriate disinfectant and leave for 30 minutes

Place all broken tubes and clean-up material in an appropriate container and discard by one of the waste disposal options

Mop the floor and laboratory benches with appropriate disinfectant

Diagram 4

Plan for action for limited aerosol accident in the BSC

PROCEDURE

Cover the spill immediately to prevent further aerosolisation. Use any available material, eg. paper towels, newspapers or even a laboratory coat

Soak the cover with appropriate disinfectant and completely wet the area

Leave the room for at least two hours to allow the HEPA filter system of the BSC to dilute the infectious droplet nuclei

Place all broken tubes/containers in an appropriate container and discard by one of the waste disposal options described earlier

Clean the inside of the BSC with appropriate disinfectant and mop the floor and bench tops

Diagram 5

Plan of action for large volume aerosol accident in the BSC

PROCEDURE

Evacuate the room immediately

Leave the BSC operating and do not re-enter the room for at least four hours. This will provide considerable dilution of the infectious droplet nuclei. Also, evacuation of the air through the HEPA filter system should reduce the potential of people outside the building becoming infected

Fog the room using a fogging machine and appropriate disinfectant.*
Allow the fog to settle

Decontaminate the BSC using formaldehyde as described earlier

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** 5% solution of a chlorhexidine gluconate (15 mg/ml) - cetrimide (150 mg/ml) mixture, or if not available 5% hypochlorite.*

6 WASTE DISPOSAL

No infected material should leave the laboratory except when it is properly packed for transport to another laboratory. All pathological material, smears, cultures and containers should at least be disinfected and preferably sterilised before disposal or re-use. *Sterilisation* means the complete destruction of all organisms, while *disinfection* implies the destruction of organisms causing disease. Sterilisation is usually accomplished by heat and disinfection by treatment with chemicals.

6.1 Discarding contaminated laboratory supplies

A container with appropriate disinfectant should be present in the BSC into which used, contaminated pipettes, loops and grinding vessels should be placed. The container should be deep enough to ensure that discarded items are covered completely. Used pipettes, wire loops etc. should be soaked for two hours after which they can be washed, sterilised and re-used.

In the immediate proximity of the BSC there should be stainless steel buckets with lids, ready to receive discarded specimen containers and tubes with bacterial suspensions.

Contaminated fluids should not be poured down drains but discarded into autoclavable containers.

Glassware should be substituted with plastic whenever possible. Broken glassware should be removed by a brush and dustpan, tongs or forceps and decontaminated in an appropriate disinfectant before disposal.

Sputum containers

Plastic sputum containers should be disposed of by incineration. Used glass sputum containers can be recycled after boiling for 20 minutes, or preferably autoclaving at 121°C and thorough washing.

Applicator sticks, pipettes, wire loops

Wooden applicators and paper should be disposed of by incineration. Contaminated or used pipettes and wire loops should be soaked for two hours in a bactericidal solution, washed and sterilised before re-use.

Positive and negative slides

Positive slides should be broken and burnt/buried to prevent their re-use. Negative slides could be re-used after proper cleaning.

6.2 Autoclaving

Autoclaving is the optimal initial sterilisation procedure and staff should be carefully instructed in the correct procedure. Ideally, the autoclave should be

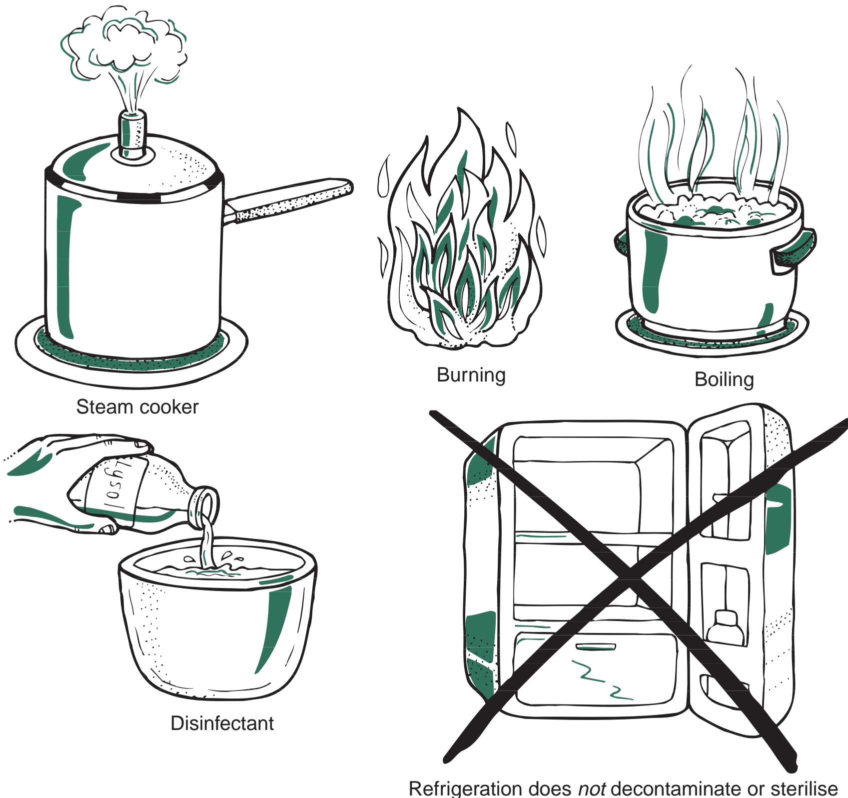
inside the tuberculosis laboratory to prevent contaminated material from being discarded or washed before decontamination. Autoclaves should be tested periodically to ensure that chamber temperatures are high enough to kill all micro-organisms. Testing can be done by steriliser indicator tubes that change colour during an adequate sterilising process.

Articles should be autoclaved at a minimum temperature of 121°C for a minimum period of 15 minutes. Autoclavable disposal bags usually contain indicator strips that change colour to indicate adequate sterilisation. After autoclaving waste material may be burned or buried. Re-usable articles may be washed and re-sterilised

6.3 Boiling and burning

Peripheral laboratories may not have autoclaves and an alternative must be provided for the disposal of specimen containers and other items. The simplest methods for treating infected material are boiling and burning. A domestic pressure cooker can be used in much the same way as an autoclave, although its capacity is limited. Alternatively, a boiler adapted from an oil-drum or petrol-can, can be suspended over a wood fire and infectious material boiled for 60 minutes before washing or discarding by burning.

Figure 2
Waste disposal



7 QUALITY CONTROL, QUALITY IMPROVEMENT AND PROFICIENCY TESTING

Quality assurance with regard to tuberculosis bacteriology is a system designed to continuously improve the reliability, efficiency and use of tuberculosis laboratory services. The purpose of a quality assurance programme is to improve the efficiency and reliability of laboratory services. In order to achieve the required technical quality in laboratory diagnosis, a continuous system of quality assurance needs to be established. Intermediate laboratories should supervise the peripheral network, while the central or reference laboratory should supervise the intermediate network.

The components of a quality assurance programme are:

- quality control
- quality improvement
- proficiency testing

7.1 Quality control

Quality control is a process of effective and systematic monitoring of the performance of bench work in the tuberculosis laboratory against established limits of acceptable test performance. Quality control ensures that the information generated by the laboratory is accurate, reliable and reproducible and serves as a mechanism by which tuberculosis laboratories can validate the competency of their diagnostic services.

Quality control is the responsibility of all laboratory workers

The specific aspects of quality control for microscopy and culture procedures are discussed extensively in the Technical Series on Microscopy and the Technical Series on Culture.

7.2 Quality improvement

Quality improvement is a process by which the components of tuberculosis laboratory services are analysed continuously to improve their reliability, efficiency and utilisation. It has been shown that the most effective and long-lasting improvements are achieved by anticipating and preventing problems rather than by identifying and correcting defects after they have occurred. Data collection, data analysis and creative problem-solving are the key components of this process. It involves continuous monitoring, identification of defects, followed by remedial action to prevent recurrence of problems. Often, problem-solving can be done efficiently only during on-site supervisory visits. These are the quickest and most effective form of quality improvement because of the personal contact and permits on the spot corrective action. Supervision should always be done by a more experienced laboratory technician. Activities during these visits should include the following:

- observing general laboratory hygiene and safety practices
- checking that written standard operating procedures for equipment and laboratory methods are in place and easily accessible
- checking that service and maintenance records of equipment are up to date
- evaluating the proportion of unsatisfactory specimens, including those where specimen containers have been reported to be broken or leaking. Should a particular health facility be identified as a source of the problem, the necessary consultation with health care staff should take place
- checking that stains and reagents and culture media contain the necessary information on preparation and expiry dates and that expired stock is not in use
- checking that positive and negative controls are used as necessary during microscopy, culture or identification procedures
- analysing the monthly proportion of positive smear or culture results for deviations from the norm
- collecting a selection of positive and negative slides for re-checking. One of the following procedures may be chosen by the national programme:
    - For every 100 slides examined, select all positives in the order in which they have been found. Next, select a collection of negative slides in random fashion, eg.

      Suppose 10 positive slides (10%) were found, select 10% of negative slides by selecting every *tenth* slide (100/10 = 10). Suppose 25 positive slides (25%) were found, select 25% of negative slides by selecting every *fourth* slide (100/25 = 4).
    - Select all positives and the following negative slide.
    - Keep all the slides for two months. If requested by the supervising laboratory, send all positive and negative slides. The selection of a proportion to be checked will be done at the supervising laboratory, or by the supervisor during a visit.
- it is particularly important to collect slides from follow-up investigations since these are more likely to contain errors (false negative results).
- evaluating the monthly culture contamination rate in terms of the proportion of specimens contaminated as well as the proportion of cultures (bottles or tubes) contaminated

A model laboratory evaluation form is presented in Annex 5.

It is essential that feedback be provided to the laboratory staff and that recommendations be made to immediately correct any deficiencies found. Training is usually very helpful in correcting deficiencies.

7.3 Proficiency testing

Proficiency testing, which is called external quality assessment by WHO standards[26, 27] refers to a system of retrospectively and objectively compared results from different laboratories by means of programmes organised by an external agency, such as a reference laboratory. The main objective is to establish between-laboratory comparability, in agreement with a reference standard. For this purpose, material for testing is prepared by the central or reference laboratory and distributed to lower level laboratories. The recipients perform the necessary procedures and report their results to the central or reference laboratory which can then assess proficiency. Detection of deficiencies through this indirect system will then determine the need for quality improvement.

Proficiency testing is highly desirable but not easy to achieve. In order to be successful they must run in the form of a continuous assessment and they require skilled and dedicated staff. The feasibility and they require skilled and dedicated staff. The feasibility and methodology of these programmes in developing countries is controversial. Nevertheless, the assurance of smear microscopy and/or culture quality is of utmost importance to National Tuberculosis Programmes.

Although quality improvement is the quickest and most effective form of (external) quality assurance, it is often difficult to perform on a regular basis owing to limitations of time and travel. Indirect technical and administrative control through proficiency testign programmes (also called "external quality assessment" or "interlaboratory test comparison") should therefore become an essential component of quality assurance.

Participation in proficiency testing programmes may be compulsory, as in hierarchical laboratory organizations, where the central or reference laboratory is responsible for those at the lower levels. It may otherwise be voluntary, where a specific quality control laboratory (QCL) of the public health laboratory service provides a variety of material to any interested laboratory. Each laboratory has a unique identification code known only to itself and the QCL. The QCL sends out collective reports which enable each of the participating laboratories to compare its proficiency to those of the others. Irrespective of whether proficiency testing programmes are compulsory or voluntary, the following (minimum) activities are recommended; preferably every six months:

7.3.1 *Microscopy*

An artificial set of standard smears with known results are sent to peripheral level laboratories. These should consist of two sets of stained and unstained smears. A minimum of five slides per set is required, covering the full range from negative to strongly positive, as follows:

Negative	:	Two slides
<10 acid-fast bacilli	:	One slide
1+	:	One slide
2+ or 3+	:	One slide

7.3.2 Culture

An artificial set of sputum specimens with known results are sent to regional or central laboratories. One set of two specimens each should be inoculated with *M. tuberculosis* and a second set of two with *M. fortuitum*, which resembles *M. tuberculosis* in terms of colony morphology and reduces nitrate but is a rapid grower that is niacin negative. A third set of two specimens should be negative.

It should be realised that results from proficiency testing programmes may be biased, since laboratory staff are aware of the origin of proficiency smears and cultures and may dedicate more time and attention to their correct processing and examination. In addition, this method does not allow measuring the quality of smear preparation. One way to overcome this problem is to send specimens (rather than smears or cultures) to participating laboratories. These could be prepared artificially by adding 1 emulsified egg to 1 000ml of 1% (w/v) aqueous methylcellulose and distributing 3.5ml volumes into specimen containers. These resemble slightly purulent sputum and are then inoculated with standardised concentrations of *M. tuberculosis* and *Nocardia asteroides* (for microscopy) or *M. tuberculosis* and *M. fortuitum* (for culture). To check on decontamination and digestion procedures, specimens could be inoculated with *Escherichia coli* as a contaminant. Clinical specimens from patients (rather than artificial specimens) can also be used and inoculated as described before.

Results should be analysed in 2x2 tables as disagreement on negative readings (false positivity), disagreement on positive readings (false negativity) and disagreement as a function of bacillary concentration (1-10 AFB, 1+, 2+, 3+). Results should be reported to the central / reference laboratory within an agreed period of time.

Irrespective of the methods of proficiency testing, the most important aspect is regular feedback and corrective measures taken in a spirit of mutual trust and agreement.

In summary, well-designed and properly managed quality assurance programmes are an asset to any laboratory. Positive aspects of such programmes for tuberculosis bacteriology include the following:

- Potential problems in the isolation and identification of *M. tuberculosis* can be greatly reduced by monitoring media and reagents before using them on clinical specimens
- Serious and costly breakdowns of equipment may be minimised by routine monitoring and maintenance
- Laboratory reports can be more accurate and expeditious as the use of inadequate media, equipment and techniques is minimised
- The quality assurance programme can serve as a learning exercise, enabling the recognition and identification of problem areas that might otherwise have been overlooked
- A good quality assurance programme will enhance the credibility of the laboratory to outside clients

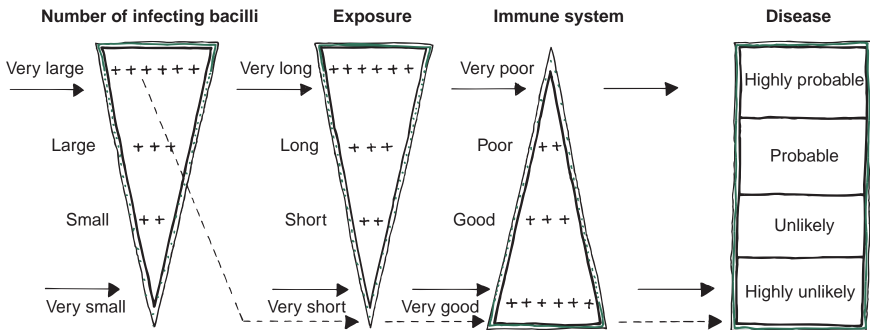
LABORATORY WORKER HEALTH MONITORING

Transmission of tuberculosis - including multidrug-resistant tuberculosis - is a recognised risk for laboratory workers, while infection with HIV facilitates transmission of tuberculosis. Most people do not develop tuberculosis disease following infection, since specific cell-mediated immunity usually develops within a few weeks after the initial infection. In immuno-competent persons, this immunity arrests multiplication of bacilli and averts clinical disease. HIV, however, kills T-helper cells (T4 lymphocytes) which reduces an infected individual defence against *M. tuberculosis*. HIV infection therefore increases the risk of reactivation of dormant tuberculosis infection, as well as the risk of progressive disease following new infection.

For the establishment of a tuberculosis infection sufficient to produce disease, exposure must be close and prolonged, the environment laden with infectious droplet nuclei and the prospective host unprotected by his/her own immune mechanisms, as indicated in Figure 3. All of these may be present in tuberculosis laboratories: Poor laboratory hygiene and disregard of safety measures increase the ease of transmission of *infection*, while factor adversely affecting the immune status of individuals (eg. HIV, diabetes, cancer, alcohol abuse) increase the development of *disease*.

Figure 3
Probability of developing tuberculosis following infection: Influence of the number of infecting bacilli, the duration of exposure and the competence of an individual's immune system

(Acnowledgement: Crofton J., Horne N., Miller F. - Clinical Tuberculosis. Butterworths, London, 1998).



Staff employed in tuberculosis laboratories should be selected carefully; they should be physically and mentally capable and should accept the responsibility for practising a healthy lifestyle. Training in laboratory procedures and strict adherence to safety measures should be accompanied by a simple surveillance programme whereby the health status of laboratory staff is monitored regularly.

8.1 Disease monitoring programme for laboratory workers

Each laboratory worker should have a confidential disease monitoring file in which screening procedures for tuberculosis as well as other health-related data are recorded. The elements of a disease monitoring programme include the following:

8.1.1 *Pre-employment profiles and baseline screening of laboratory workers*

A standardised health questionnaire should be completed for each employee. This questionnaire should relate past tuberculosis infection disease, BCG vaccination status, underlying medical conditions which may compromise the susceptibility to tuberculosis and previous contact with confirmed tuberculosis cases. A model structured health questionnaire is provided in Annex 6.

A baseline chest x-ray and a Mantoux tuberculin skin test (TST) should be performed. Strongly positive reactors with skin test diameters of >15mm and symptoms suggestive of tuberculosis should be evaluated clinically and microbiologically. Two sputum specimens, collected on successive days, should be investigated for tuberculosis by microscopy and culture.

Confidential HIV testing with pre-and post-test counselling should be offered to all laboratory workers. BCG re-vaccination as a means of preventing tuberculosis in laboratory workers is not recommended.

8.1.2 *Quarterly monitoring of health status*

Laboratory workers should declare information on their health status in the form of answers to specific questions relating to the early signs and symptoms of TB. These include cough for longer than three weeks, weight loss, anorexia, night sweats and the frequent occurrence of colds or other respiratory infection episodes in recent weeks.

The laboratory worker's weight should be recorded during each monitoring exercise and an unexplained loss of more than 10% during the previous quarter should be followed up with clinical and microbiological investigations for tuberculosis.

Quarterly information on health status can be obtained by using a single structured questionnaire, as illustrated in Annex 6.

8.1.3 *Post-exposure monitoring*

Following an accident in the laboratory the laboratory workers" health monitoring file should be reviewed. S/he should be carefully monitored clinically. Eight weeks after the exposure episode a chest x-ray examination should be performed, together with a TST in cases where the baseline reaction diameter was <10mm.

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ANNEX 1

ASSESSMENT TO ASCERTAIN WHETHER THE NUMBER OF MICROSCOPY CENTRES IN A COUNTRY IS ADEQUATE

As an example, consider a hypothetical high prevalence country with a total population of 3 million. The incidence of smear positive tuberculosis is estimated to be 90 per 100 000 of the total population, while ten suspects will have to be screened to detect one smear positive case. On average, six smear examinations will be done on every smear positive case, viz three at diagnosis, one after two months of therapy, another after three months of therapy and the last one at the end of treatment. The country has 30 existing microscopy centres.

Example

Total population	:	3 000 000
Smear positive incidence	:	90 per 100 000 population
Proportion of smear positive among suspects	:	10%
Average number of smears per case	:	6

1. the *total number of smear positive cases* that can be expected is:

90/100 000 x 3 000 000 = 2 700

2. Ten suspects screened for every case identified and three smears are done per suspect. Therefore, to detect 2 700 smear positive cases will require:

   2 700 x 10 x 3 = 81 000 smears for screening

3. Every smear-positive case will have three additional smear-examinations for control:

   2 700 x 3 = 8 100

4. The total number of smears to be done in one year, will therefore be:

81 000 (suspects) + 8 100 (follow-up of cases) = 89 100

5. The average number of working days in a year is 250. This means that an average of 325 smears will be done per working day: 89 100 / 250 = 325

6. There are 30 existing microscopy centres. On average 11 smears are done per centre (325 / 30), which is well within the recommended range of 2-20 per microscopist per day.

ANNEX 2a

LABORATORY REQUEST FORM FOR MICROSCOPY

Name of Health Centre ____________________________ Date ______________

Name of patient _______________________________ Age _____ Sex M ❐ F ❐

Complete address:____

____

Patient's register number* ______________________

Source of specimen ❐ Pulmonary
❐ Extra-pulmonary Site _____________________

Reason for examination ❐ Diagnosis
❐ Follow-up of chemotherapy

Specimen identification number _________________ Date ________________
Signature of person requesting examination ______________________________

** Be sure to enter the register number for the follow-up of patients on chemotherapy*

ANNEX 2b

LABORATORY REQUEST FORM FOR CULTURE

Name of Health Centre _____________________________ Date ______________

Name of patient ________________________________ Age _____ Sex M ❒ F ❒

Complete address:____

____

Patient's register number* _______________________

Source of specimen ❒ Pulmonary
❒ Extra-pulmonary Site ______________________

Reason for examination ❒ Diagnosis
❒ Follow-up of chemotherapy

Specimen identification number _________________ Date _________________
Signature of person requesting examination _______________________________

** Be sure to enter the register number for the follow-up of patients on chemotherapy*

LABORATORY REGISTER FOR MICROSCOPY

Lab Serial no.	Date .	Name (in full)	Sex M/F	Age	Complete adress (for new patients)	Name of referring Health Centre	Reason for examination*		Microscopy Results			Signature	Remarks
							Diagnosis	Follow-up	1	2	3		

* If sputum is for diagnosis, put a tick (✓) mark in the space under "Diagnosis"
If sputum is for follow-up of patients on treatment, write the patient's register number in the space under "Follow-up".

LABORATORY REGISTER FOR CULTURE

Lab Serial no.	Name (in full)	Sex M/F	Age	Complete adress (for new patients)	Name of referring (for new patients)	Mycroscopy results	Culture results	Colony morphology	Growth rate	Niacin	Catalase	Ziehl-Neelsen confirma-tion	Signature	Remarks
										Nitrate	PNB			

MICROSCOPY RESULTS

Laboratory serial number: ____________________________

Visual appearance of sputum

	Mucopurulent	Blood-stained	Saliva
Specimen 1	❒	❒	❒
Specimen 2	❒	❒	❒
Specimen 3	❒	❒	❒

Microscopy results

Staining method ❒ Ziehl-Neelsen ❒ Fluorochrome

Date	**Specimen**	**Results***	**Positive (grading)**			
			3+	**2+**	**1+**	**1-9 AFB**
		1				
		2				
		3				

**Write negative or positive*

Date: _____________________ Examined by (signature): __________________

ANNEX 4a

CULTURE RESULTS: PRELIMINARY REPORT

Laboratory serial number ______________ Date specimen received ___________

0Culture results

Culture method ______________________

No growth	❐	3+	❐
1-19 colonies	❐	4+	❐
1+	❐	Contaminated	❐
2+	❐		

Cultivation yielded _____________ growth of mycobacteria with the characteristics of tubercle bacilli.

A final report will be issued within the next four weeks.

Date ___________________________ Signature ______________________

ANNEX 4b

CULTURE RESULTS: FINAL REPORT

Laboratory serial number ______________ Date specimen received _______________

Microscopy results

Staining method ❐ Ziehl-Neelsen ❐ Fluorochrome

Negative	❐	1+	❐
Not done	❐	2+	❐
1-9 AFB	❐	3+	❐

Culture results

Culture method ______________________

No growth	❐	1-19 colonies	❐
Contaminated	❐	1+	❐
Not done	❐	2+	❐
		3+	❐
		4+	❐

Culture identification

Growth rate__________________________ Colony morphology __________________

Niacin production	❐ positive	❐ negative
Nitrate production	❐ positive	❐ negative
Other, list___________	❐ positive	❐ negative
___________	❐ positive	❐ negative

Culture identified as	*Mycobacterium tuberculosis*	❐
	MOTT	❐

Date _____________________________ Signature _____________________________

ANNEX 5

EVALUATION FORM: EXTERNAL LABORATORY QUALITY ASSURANCE

Laboratory name and address

Date of evaluation

d	d	/	m	m	/	y	y
		/			/		

GENERAL ASPECTS

	Adequate	*Inadequate*
Laboratory supplies and reagents	❐	❐
Laboratory equipment	❐	❐
Biosafety measures	❐	❐

TECHNICAL ASPECTS

Microscopy	*Good*	*Fair*	*Poor*
Smear preparation	❐	❐	❐
Staining procedures	❐	❐	❐
Examination procedures	❐	❐	❐
Recording and reporting of results	❐	❐	❐
Storage of slides for quality control	❐	❐	❐

Proportion of positive smears per month ______ %

Culture and identification	*Good*	*Fair*	*Poor*
Digestion / decontamination procedures	❐	❐	❐
Inoculation and incubation procedures	❐	❐	❐
Reading of cultures	❐	❐	❐
Confirmation of M. tuberculosis	❐	❐	❐
Recording and reporting of results	❐	❐	❐

Proportion of positive cultures per month ______ %

Proportion of contaminated cultures per month ______ %

Proportion of contaminated specimens per month ______ %

ADMINISTRATIVE ASPECTS

	Complete	*Incomplete*	
Standard operating procedures	❒	❒	❒
Laboratory register	❒	❒	❒
Laboratory reports	❒	❒	❒
Laboratory accident book	❒	❒	❒

Calculation of false positive and false negative results

Reference result

		Positive	**Negative**	**Total**
Test result	**Positive**	a	b	(a+b)
	Negative	c	d	(c+d)
	Total	(a+c)	(b+d)	(a+b+c+d)

% False positive = [b/(a+b)] x 100
% False negative= [c/(c+d)] x 100

Recommendations

____
____
____

Corrective measures taken______________________
____
____

______________________________________ ______________
Name and signature of supervisor Date

______________________________________ ______________
Name and signature of laboratory technician/technologist Date

TUBERCULOSIS STAFF HEALTH QUESTIONNAIRE

Name ______________________________ Age ☐☐ yrs Date of appointment ☐☐/☐☐/☐☐

Laboratory Address ____

Baseline screening:

Previous BCG vaccination No | Yes if yes, year ☐☐☐☐ Outcome

- ☐ Cured
- ☐ Treatment completed
- ☐ Treatment interrupted
- ☐ Treatment failed

Signs and symptoms of tuberculosis

	No	Yes		No	Yes		No	Yes
Cough > 3 weeks			Chest pain			Lethargy		
Weight loss			Anorexia			Night sweats		

Other illnesses

____

____

____

	Mantoux test			Chest X-ray		Sputum investigation				Weight (kg)
	Date	Tu	Result (mm.)	Date	Result	Date	Mycroscopy result	Culture result	Drug susceptibility result	
Baseline Follow-up										

HIV test offered No | Yes accepted No | Yes

Quarterly health monitoring

Irst quater

	No	Yes		No	Yes		No	Yes
Cough > 3 weeks			Chest pain			Lethargy		
Weight loss			Anorexia			Night sweats		

Other illnesses

_______________Date_______

_______________Date_______

_______________Date_______

Weight

Kg

Second quater

	No	Yes		No	Yes		No	Yes
Cough > 3 weeks			Chest pain			Lethargy		
Weight loss			Anorexia			Night sweats		

Other illnesses

_______________Date_______

_______________Date_______

_______________Date_______

Weight

Kg

Third quater

	No	Yes		No	Yes		No	Yes
Cough > 3 weeks			Chest pain			Lethargy		
Weight loss			Anorexia			Night sweats		

Other illnesses

_______________Date_______

_______________Date_______

_______________Date_______

Weight

Kg

Fourth quater

	No	Yes		No	Yes		No	Yes
Cough > 3 weeks			Chest pain			Lethargy		
Weight loss			Anorexia			Night sweats		

Other illnesses

_______________Date_______

_______________Date_______

_______________Date_______

Weight

Kg

ANNEX 7

UNIT CONVERSION FACTORS LENGTH

1mm	=	0.0394 inch	1 inch	=	25.4mm
1m	=	39.37 inches	1 inch	=	0.0254m
1m	=	3.28 feet	1 foot	=	0.305m
1m	=	1.09 yard	1 yard	=	0.91m

TEMPERATURE

0°C	=	(°F-32) x 5/9
°F	=	(°C x 9/5) + 32
100°C	=	212°F

WEIGHT

1g	=	0.035 ounce	1 ounce	=	28g
1kg	=	2.2lb	1lb	=	0.454kg

AREA

$1cm^2$	=	$0.155in^2$	$1m^2 = 10^4\ cm^2 = 10.76ft^2$
$1in^2$	=	$6.452cm^2$	$1ft^2 = 144in^2 = 0.0929m^2$

VOLUME

1 litre = $1000cm^3 = 10^{-3}\ m^3 = 0.0351ft^3 = 61.02in^3$ 500ml = 16fl oz = 1pint
$1ft^3 = 0.02832m^3$ = 28.32 liters = 7.477 gallons 1 000ml = 1 liter = 33.8fl oz = 1 quart
1 US gallon = 3.78ℓ 1UK gallon = 4.55ℓ 1 000ml = 0.22 UK gallon = 0.26 US gallon
1ml = 1cc = 0.034fl oz

VELOCITY

1cm / s = 0.03281 ft/s 1ft/s = 30.48cm/s
1km/h = 0.2778 m/s

PRESSURE

1Pa = $1N/m^2 = 1.451 \times 10^{-4}\ lb/in^2 = 0.209\ lb/ft^2$
$1lb/in^2$ = 6 891Pa $1lb/ft^2$ = 47.85Pa
1atm = 1.013×10^5Pa = $14.7lb/in^2$ = 2117 lb/ft

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