Quality Assurance of Sputum Microscopy in DOTS Programmes

Regional Guidelines for Countries in the Western Pacific

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Stop TB Special Project

Authorship

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Preface

Tuberculosis control by Directly Observed Treatment, Short-course (DOTS) has been introduced in many countries in the Western Pacific Region. DOTS is the proven, cost-effective strategy recommended by World Health Organization for countries with limited resources.

Laboratory diagnosis of active tuberculosis cases by sputum smear microscopy is a critical element of DOTS – to the extent that the quality of the tuberculosis laboratory service has a major influence on the success of the tuberculosis control programme. It follows that tuberculosis control will be most effective (and efficient) in countries that have a network of laboratories providing a reliable service within the framework of the National Tuberculosis Programme. Improvement of laboratory services throughout the Western Pacific Region is now a priority of the Stop Tuberculosis initiative.

Regardless of its purpose, a reliable laboratory service is one that is cost-efficient and provides results that are consistently accurate. These demands can be met only through commitment to quality assurance. A key component of quality assurance for tuberculosis microscopy services is External Quality Assessment – the process by which the performance of a routine diagnostic service is monitored by a more competent laboratory such as a reference laboratory.

A committee of representatives from various global authoritative bodies has recently prepared a comprehensive guide on External Quality Assessment for DOTS laboratories: "External Quality Assessment for AFB Smear Microscopy". The document has been reviewed and further developed by World Health Organization, Western Pacific Region.

These Regional Guidelines have been prepared to guide both laboratory and National Tuberculosis Programme staff in strengthening quality assurance of sputum smear microscopy. The aim is to improve the quality of the National Tuberculosis Programme in the Western Pacific Region. The guidance offered here draws on the international guidelines for External Quality Assessment, but includes additional information, in particular on quality control, to ensure routine monitoring of all aspects of laboratory activity.

It is hoped that these quality assurance guidelines will be adopted and implemented in the Region as a means of improving and sustaining the high quality of the National Tuberculosis Programme.

Abbreviations

AFB	acid-fast bacillus (or bacilli)
DOTS	Directly Observed Treatment, Short-course
EQA	external quality assessment
IUATLD	International Union Against Tuberculosis and Lung Disease
LQAS	Lot Quality Assurance System
NRL	National Reference Laboratory
NTP	National Tuberculosis Programme
OIF	oil immersion fields
QA	quality assurance
QC	quality control
QE	quantification error
SPR	slide positivity rate
TB	tuberculosis
WHO	World Health Organization
WPR	WHO Western Pacific Region
ZN	Ziehl-Neelsen stain

1 Introduction

1.1 Basic concepts of quality assurance in pathology laboratories

In a broad sense, a pathology laboratory can be regarded as a factory producing results of tests on clinical samples taken from patients at the request of medical staff. Clearly, the quality of the laboratory product is critical to the treatment of the patient.

The most important element in the quality of the test result is its *accuracy* (or correctness). If the laboratory result is falsely negative, there is a chance that the patient's illness will go undiagnosed, or, in some cases, will be incorrectly diagnosed. Possible consequences for the patient include continued suffering, or even death. If the patient happens to be suffering from an infectious disease, there is a risk of continued transmission to the patient's family and close contacts. If the laboratory result is falsely positive, there will be an incorrect diagnosis and the patient is likely to receive unnecessary treatment, such as hospitalization and therapy with toxic drugs.

Accuracy is understandably the element of quality that is given most attention. However, in addition to accuracy – which can be measured by various means – there is a need to consider many other aspects of the laboratory's operation. These include:

- Is the laboratory environment appropriate for the work being performed?
- Are the staff numbers adequate for the workload?
- Are the operating procedures up-to-date and followed by all staff?
- Are all staff adequately trained in the test processes?
- Are the results produced economically?
- Is the laboratory working in collaborative partnership with its clients, the medical staff?

In order to demonstrate and maintain high quality results, a laboratory's overall performance should be monitored through a series of regular activities. In combination, such activities make up the laboratory's quality assurance (QA) programme.

Quality assurance activities take many forms, some of which are common to all pathology laboratories, regardless of the tests performed. They include:

- validation of samples submitted for testing;
- regular checking of reagents used in test procedures (including expiry dates);
- inclusion of standards (or samples of known positivity) in routine test runs;
- periodic review and updating of procedure manuals;
- regular maintenance and calibration of equipment;
- data collection and analysis;
- regular meetings with the laboratory's clients.

Quality assurance activities produce various pieces of information: some will be quantitative (e.g. rate of errors in a test panel), and some will be qualitative (e.g. poor maintenance of the microscope). The combined results serve as a rational basis on which the performance of the laboratory can be assessed. Some QA exercises will allow inter-laboratory comparison of individual performance. While the accuracy of results is of major interest to staff, managers and clients, an important consequence of QA activities is the corrective action that must occur when deficiencies are identified. It follows that laboratories providing external quality assessment (EQA) must have the expertise to make recommendations that will rectify any identified problems. The most effective QA exercises are those that detect problems *before* they have any impact on the reported result. Thus, QA should be seen as a prospective as well as a retrospective activity. The cyclical process of regular application of QA exercises leading to corrective actions is known as quality improvement.

In order for QA to be fully effective, all information obtained in QA exercises, as well as any consequential corrective actions, must be recorded and filed to enable periodic review by supervisors and managers. For EQA, it is essential that results are communicated in writing and the report must include a summary of findings and any relevant recommendations. Finally, it is essential that all laboratory personnel have a clear understanding of the principles of QA, and be fully informed on the laboratory's performance.

1.2 Rationale for quality assurance of sputum smear microscopy in TB DOTS programmes

The microscopy result is used to categorize the patient by the standard definition of DOTS (Directly Observed Treatment, Short-course). Almost always, a *positive* smear will indicate that the patient has active tuberculosis. The result must be communicated immediately to supervising medical staff within the National Tuberculosis Programme (NTP). Furthermore, patients with sputum smear-positive tuberculosis should immediately commence anti-tuberculosis treatment because they are infectious to contacts (family, friends, workmates). The initial period of treatment is usually referred to as the intensive phase. Treatment will rapidly render the patient non-infectious and reduce the symptoms of tuberculosis (cough, weakness, lethargy, etc). A *negative* smear will usually mean that a patient is unlikely to transmit tuberculosis to contacts – and in fact may have a disease other than tuberculosis. However, note that in the event of a negative smear result, further tests and clinical evaluation of the patient may result in the patient being diagnosed as a case of sputum smear-negative tuberculosis.

In addition to diagnosing (or excluding) active tuberculosis, microscopy has a further use: medical staff rely on the smear for monitoring progress of patients with sputum-smear positive tuberculosis whilst they are receiving anti-tuberculosis treatment. Samples collected during treatment are referred to as follow-up specimens. As a general rule, a *negative* smear at the end of the intensive phase of treatment (after two months) confirms that the patient's infection is responding to the drug regimen. The patient then enters the continuation phase (in which only two drugs are used). On the other hand, a *positive* smear during treatment suggests that the patient may still be infectious (or may have drug-resistant tuberculosis). In such cases, the intensive phase of therapy will usually be extended by at least one month. Finally, the microscopy result from a specimen collected at the end of treatment is used to confirm the cure of the patient. The cure rate is one of the most important performance indicators for the NTP.

Such is the importance of microscopy, it follows that errors will be highly significant – not only for the patient but also for the NTP. TABLE 1 (page 4) summarises the consequences of false (incorrect) results in sputum smear microscopy. Note the different consequences, depending on whether specimens are for diagnosis or follow-up.

(In this context a false-negative result refers to a *negative* microscopy report for a patient who has tuberculosis. A false-positive result occurs when a *positive* report is issued for a patient who does not have tuberculosis.)

However, for reasons outlined above, the main focus of QA programmes for sputum smear microscopy will be the *reliability* of the smear result. It is therefore essential that QA programmes:

Table 1 **Consequences of false results in sputum smear microscopy**

Diagnostic specimens		Follow-up specimens	
False-negative	**False-positive**	**False-negative**	**False-positive**
Patient continues to suffer symptoms of TB	Patient is incorrectly recorded as TB case	Patient is incorrectly recorded as "cured"	Patient is incorrectly recorded as "treatment failure"
Patient continues to transmit TB to contacts	Patient will receive unnecessary drug treatment, hospitalization	Treatment may be discontinued prematurely	Patient may receive unnecessary drug treatment
Patient may be incorrectly categorized as smear-negative TB	NTP resources are wasted	Missed failure cases may cause transmission of drug-resistant organisms to contacts	NTP resources are wasted

- ensure that the reported results are accurate;
- identify any practices that are potential sources of error;
- ensure that appropriate corrective actions are initiated.

The format of QA programmes in sputum smear microscopy reflects the many and varied sources of error. Laboratory staff at all levels – in particular those responsible for supervision – must have a full understanding of the many factors that can lead to false results. TABLE 2 shows the common sources of false results in sputum smear microscopy. As the table shows, errors can arise *before* the sample reaches the laboratory. While the laboratory is not directly responsible for such errors, its QA programmes must have the ability to detect (and correct) such problems. Also, in the laboratory itself, errors can be due to administrative as well as technical faults.

The most effective QA programmes will be those that challenge *all* areas of the laboratory service. They must have broad coverage, checking administrative as well as technical activities, and must extend beyond the laboratory (include specimen collection and transport).

Table 2 **Common sources of false results in sputum smear microscopy**

		Result	
Location	**Category**	**False negative**	**False positive**
Pre-laboratory	Administrative	■ specimen quality ■ patient identification ■ specimen labelling ■ transport conditions	■ specimen container ■ patient identification ■ specimen labelling
Laboratory	Administrative	■ specimen handling ■ specimen registration ■ recording and/or reporting result	■ specimen registration ■ recording and/or reporting result
	Technical	■ smear preparation ■ stain formulations ■ staining technique ■ microscope performance ■ smear examination technique	■ smear preparation ■ stain formulations ■ staining technique ■ smear examination technique

NB: In this context, a "false" result is one that disagrees with the true clinical situation.

1.3 Consensus document on External Quality Assessment for AFB Smear Microscopy

"External Quality Assessment for AFB Smear Microscopy (2002)" is a consensus document written by a panel of 16 laboratory experts. Its preparation was supported by Centers for Disease Control and Prevention and the Association of Public Health Laboratories, both of the United States, in collaboration with the international agencies: International Union Against Tuberculosis and Lung Disease (IUATLD), Japan Anti-Tuberculosis Association (JATA), Royal Netherlands Tuberculosis Association (KNCV), and WHO. The workgroup's mission was "to identify different methods to assess the quality and reliability of laboratory services and to provide them in a simple practical format". (Quality assessment of clinical diagnostic and treatment practices was considered beyond the scope of the work group's charge.)

The publication is primarily concerned with EQA, rather than quality control (QC) or quality improvement (the other components of QA). The authors note that "…EQA is an expansion of proficiency testing as described by IUATLD." They further state that the recommendations in the document "…are intended to replace (revise and update) the methods described in previous guidance from IUATLD and WHO."

In the introduction, the authors state:

> "Both the availability and quality of AFB smear microscopy is dependent on national programmes that support, train and monitor the testing performance of individual laboratories."

The consensus document recommends that EQA be conducted through one or more of:

- on-site evaluation (supervisory visits);
- panel testing;
- blinded rechecking.

At a meeting of WHO Western Pacific Region (WPR) laboratory personnel in Manila, April 2002, it was agreed that the EQA consensus document would be adopted as a basis for wider implementation of QA for sputum smear microscopy in WPR.

The following chapter includes recommendations on EQA for countries in WPR and discusses the advantages, disadvantages and application of the various components of EQA.

1.4 Issues affecting the implementation of EQA for sputum smear microscopy

The global targets for tuberculosis control are:

- 100% DOTS coverage;
- 70% case detection; and
- 85% treatment success.

For reasons outlined earlier, these targets cannot be met unless each country has a network of laboratories whose reliability is guaranteed through a commitment to QA activities.

In most countries in WPR with a high burden of tuberculosis, the laboratory network and QA are being gradually strengthened, along with expansion of DOTS.

International authorities have stressed that implementation of effective QA for tuberculosis microscopy requires extensive resources. It is also recognized that *sustainability* of QA activities is a major issue. To that end, it is recommended that QA is implemented in a stepwise fashion. Thorough planning is essential, and for a given country, numerous factors must be taken into account, including:

- demography;
- estimated tuberculosis incidence;
- existing DOTS programmes;
- existing laboratory network;
- human resources;
- capacity of National Reference Laboratory (NRL);
- geography and climate;
- economic situation;
- transport infrastructure.

Implementation of QA will be most simply achieved in countries (or regions) where there is already an effective tuberculosis laboratory network with strong links to the NTP. Best results will be achieved where laboratories at all levels are working under standard operating procedures as set down by the NTP. There should also be an NRL, where senior staff are highly motivated and experienced in diagnostic tuberculosis services. This laboratory should work with NTP in formulating policy, training and procurement of laboratory reagents and equipment.

2 Tools for quality assurance in sputum smear microscopy

2.1 Elements of quality assurance

As stated in the INTRODUCTION (page 1), it is essential that tuberculosis DOTS programmes are supported by a network of laboratories providing a reliable and accurate service. The National Tuberculosis Programme (NTP) will demand that laboratories at all levels are managed by a system of quality assurance (QA) that meets international standards.

WHO and International Union Against Tuberculosis and Lung Disease (IUATLD) define QA for sputum smear microscopy as follows:

- Quality control (QC) is a systematic internal monitoring of working practices, technical procedures, equipment and materials, including quality of stains.
- External quality assessment (EQA) is a process to assess laboratory performance. EQA includes on-site assessments (supervisory visits), panel testing and slide rechecking.
- Quality improvement is a process by which the components of smear microscopy diagnostic services are analyzed with the aim of looking for ways to permanently remove obstacles to success.

In this document, emphasis will be given to QC and EQA. Quality improvement is not given separate attention since it is dealt with when discussing the actions that should follow detection of problems, errors, deficiencies, etc.

2.2 Quality control

WHO manuals make the following points with respect to quality control (QC):

- QC is a process of effective and systematic *internal* monitoring of the performance of bench work.
- QC ensures that the information generated by the laboratory is accurate, reliable and reproducible. It serves as a mechanism by which tuberculosis laboratories can validate the competency of their diagnostic services by assessing the quality of specimens; by monitoring performance of microscopy procedures, reagents and equipment against established limits; by reviewing microscopy results; and by documenting the validity of microscopy methods.
- QC should be performed on a regular basis.
- For a QC programme to be of value, it must be practical and workable.
- QC is the responsibility of all laboratory workers.

(See **"Services in Tuberculosis Control, Part II: Microscopy, WHO, Geneva [1998],"** page 47.)

Many aspects of QC are either carried out in conjunction with routine testing or form part of the everyday management of the laboratory. In contrast, EQA is intended to gather information to demonstrate that QC is performed regularly, and includes activities designed to show that the reported results meet accepted standards.

Quality control in laboratories performing sputum smear microscopy is logically divided into:

- administration;
- specimen submission; and
- microscopy.

TABLES 3, 4 (page 10) and 5 (page 11) set out the standards and checks that should be applied to the performance of sputum smear microscopy for tuberculosis.

Table 3 **Elements of quality control – administration**

Sub-section	Standards	Quality control checks
Workplace	A. Tuberculosis microscopy should be performed in a secure, dedicated work space. B. The laboratory should be organised to allow efficient flow of work.	■ work areas should be clean, tidy, free from unused equipment, and set out as suggested in relevant manuals. ■ the laboratory should be cleaned and tidied at the end of each working day. ■ unauthorized access must be restricted during working hours. ■ the laboratory should be locked outside working hours.
Staffing	A. Staff must have technical knowledge and skills appropriate for laboratory work. B. Staff must have received training in sputum smear microscopy.	■ staff must have the technical knowledge and skill required for laboratory work. ■ staff must receive training in sputum smear microscopy. ■ staff must take part in regular proficiency tests and receive retraining as required. ■ each staff member must have a current training record.

Continued next page

Table 3 **Elements of quality control – administration** (Cont'd)

Sub-section	Standards	Quality control checks
Standard operating procedures	A. Methods must comply with current international standards (e.g. WHO manuals). B. Procedures must be written exactly as performed in the laboratory and collected in an up-to-date method manual. C. Method manuals must be located in the laboratory with easy access for all staff.	■ methods in use must be as recommended by the NTP. ■ the method manual must be located in the laboratory work area. ■ all methods must be reviewed at least annually, and alterations initialled by the supervisor and brought to the attention of all staff.
Laboratory register	A. All work performed must be recorded in standard format in the laboratory register. B. The laboratory register must be available to both laboratory and NTP staff at all times.	■ the register must be in a format approved by the NTP. ■ the register must be located in the laboratory work area at all times and stored in a secure location. ■ the register must be legible and up-to-date. ■ results should be written directly into the register rather than transcribed from a worksheet.
Data collection	A. The laboratory must collect and analyse data on workload and results.	■ a report including statistics on workload and results should be submitted to the local DOTS co-ordinator each quarter.
Equipment	A. All laboratory equipment must be maintained in safe working condition. (Sputum smear microscopy requires an electric, binocular microscope, capable of x 1000 magnification.)	■ laboratory records must show supplier, date of purchase, serial number and cost of each piece of equipment. ■ the manual should be located with the instrument. ■ staff must be trained in care and maintenance of the microscope. ■ microscopes must be cleaned daily and stored in a dry environment (where practicable). ■ equipment must be serviced as recommended by the manufacturer, and service records must be kept in the laboratory.
Supplies	A. The laboratory must have a reliable system for ordering, delivering and maintaining stocks of supplies.	■ the system for ordering and delivery of supplies must ensure there are no delays in testing. ■ laboratory staff must take responsibility for ensuring there are adequate stocks of stains, slides, etc. ■ where practicable, there must be buffer stocks to allow for interruptions in supply.
Laboratory safety	A. Safety in the workplace is a responsibility shared by both employer and employee. (Sputum smear microscopy for tuberculosis is a low-risk procedure when performed by trained personnel in a ventilated work area. Safety cabinets are not necessary. Face masks and gloves have limited value.)	■ staff must be in good general health and aware of the symptoms of tuberculosis. ■ staff must have ready access to medical services for tuberculosis investigations (if required). ■ staff must have sound knowledge of bio-safety as it applies to laboratory testing for tuberculosis. ■ laboratory coats must not be worn outside the work area. ■ a freshly prepared tuberculocidal disinfectant must be available at all times. ■ the laboratory should be well ventilated, particularly during smear preparation and Ziehl-Neelsen staining. ■ safety cabinets should not be used in rooms with open doors, open windows, or ceiling fans. ■ the microscopy bench must be of suitable height and design; stools should have back supports. ■ staff must be informed on other hazards in the laboratory (chemical, electrical, mechanical).

Table 4 **Elements of quality control – specimen submission**

Sub-section	Standards	Quality control checks
Collection	A. Sputum samples must be collected in containers that are clean, sterile, screw-capped, transparent and labelled. B. Strict attention must be paid to quality of specimens (sputum, rather than saliva or nasal secretions).	■ samples must be collected under guidelines endorsed by the NTP. ■ staff involved in collection of samples must receive specific training. ■ patients must receive instruction from trained personnel prior to collecting sputum. ■ the container must be labelled with patient details before sample is collected. ■ collection must take place in a ventilated area (e.g. outside). ■ persons responsible for sample collection must check the quality of the sample before accepting and forwarding to the laboratory. ■ a completed request form must accompany specimens during transport to the laboratory.
Transport	A. Samples must be forwarded to the laboratory by a secure process as soon as practicable after collection. B. Samples must not be exposed to extreme environmental conditions (heat) during transport.	■ the laboratory must have a role in making guidelines for the collection and transport of specimens from its locality. ■ where delays are unavoidable, specimens should be stored at cool temperatures.
Handling in the laboratory	A. Samples must be handled efficiently to ensure prompt and accurate reporting of results. B. Details of submitted samples must be entered into the laboratory register before tests are carried out. C. Specimen quality must be checked visually and recorded in the register before tests are carried out.	■ delivery of samples must be made to a designated location in the laboratory. ■ security of samples must be maintained at all times. ■ patient details must be matched with information on the specimen container before registration. ■ specimens that do not comply with collection guidelines should be rejected and repeat samples collected. ■ the laboratory number must be written on the side (or side and top) of the container. ■ a visual assessment of the specimen quality must be entered into the laboratory register.

Table 5 **Elements of quality control – microscopy**

Sub-section	Standards	Quality control checks
Smear preparation	A. The smear must be made from a representative portion of the specimen. B. The smear must be heat-fixed to the slide to prevent loss during staining, etc.	■ smears must be prepared on clean, unused glass slides. ■ before making the smear, the slide must be clearly labelled with the laboratory number (taking care not to contaminate the slide through finger contact, etc). ■ there must be only one smear per slide. ■ a swab-stick (or loop) must be used to collect a representative portion of the sample for smearing. ■ the smear must be approx. 2cm x 1cm in the centre of the slide. ■ after drying, fixation must be done by gentle heating over a flame. ■ the fixed smear should have the appearance of a milky-white film on the slide.
Staining	A. Staining must be done using a standard method for Ziehl-Neelsen. B. Performance of staining reagents must be checked at monthly intervals (at least).	■ the staining method must be endorsed by the NTP. ■ the staining method must be readily available (laboratory manual, wall chart). ■ all reagent bottles must be labelled, and show preparation and expiry dates. ■ performance of reagents must be checked with a known positive slide at monthly intervals (or more frequently), and results entered in the register (or special book). ■ staining sinks must be level.
Smear examination	A. The smear must be examined in accordance with WHO recommendations (representative area of the slide, at least 100 effective fields but up to 300).	■ the method for smear examination must be readily available (laboratory manual, wall chart). ■ microscope should be binocular, electric, and with good optics. ■ the microscopy bench and chair must be comfortable for the microscopist. ■ positives must be scored in accordance with WHO recommendations. ■ the objective lens must be wiped clean after use on a positive smear. ■ results should be entered directly into the laboratory register. ■ the slide (if frosted) should be signed by the technician who performed the examination. ■ all slides must be stored in sequence for re-examination by EQA.
Reporting	A. Results must be reported in accordance with WHO recommendations (in writing, new positives by telephone).	■ all results must be reported in a standard format (NTP-approved). ■ where practicable, new positives should be verified by another worker before reporting. ■ results must be reported as soon as practicable. ■ positives from new patients should be reported immediately.

2.3 External Quality Assessment

2.3.1 ON-SITE EVALUATIONS

The EQA consensus document makes the following statement:

> "A field visit is the best method to obtain a realistic picture of the conditions and practices in the laboratory; therefore, on-site evaluation of peripheral laboratories is an essential component of a meaningful EQA programme."

A major advantage of an on-site evaluation is that it involves direct contact between peripheral technicians and supervisory staff from the intermediate or central level. Furthermore, assessment of the laboratory under actual working conditions allows corrective actions to be implemented without delay. A major disadvantage of on-site evaluations is that they consume significant resources – in travel costs as well as personnel. Personnel performing on-site evaluations must possess special skills and be appropriately trained. Travel to the peripheral centres will require them to be absent from their normal place of work. (As a cost-saving measure in countries where health sector reform has been implemented, laboratory assessments for tuberculosis can possibly be incorporated with QA activities for other programmes.)

The DOTS strategy requires quarterly visits by the district DOTS supervisor to each DOTS centre, where there will usually be a microscopy laboratory (see TABLE 6). With training, the DOTS supervisor can also carry out a limited assessment of the laboratory as part of this visit. A report should be prepared for the NTP. Where significant deficiencies are found, a technician from the intermediate or central level should visit the peripheral laboratory as a matter of priority. A further important function of the visit to the peripheral laboratory is to select slides for blinded rechecking (see SECTION 2.3.3, page 15). At the same time, results of the previous round of testing can be delivered and discussed. In addition to the quarterly assessment by the DOTS supervisor,

Table 6 **On-site evaluation of peripheral laboratories**

Officer	Frequency	Main activities
District DOTS supervisor	Quarterly	■ observe general laboratory environment ■ check microscope performance ■ check level of supplies ■ collect slides for rechecking
Laboratory technician from intermediate or central level	At least annually	■ perform full assessment of laboratory
Laboratory technician from intermediate or central level	Whenever rechecking detects major errors	■ perform full assessment of laboratory

it is also recommended that a person with laboratory expertise visits peripheral laboratories at least once a year. It would be beneficial if such a visit coincided with the quarterly DOTS supervision. Obviously, EQA by on-site evaluation will be most readily implemented in countries (or regions) where DOTS is well established and supported by a structured laboratory network.

In summary, it is recommended that on-site evaluations in countries in the Western Pacific Region take the form of a visit by:

1) a member of the DOTS supervision team each quarter
2) a technical/scientific officer, usually in conjunction with the DOTS supervision team (quarterly, or at least annually)
3) a technical officer in response to detection of major errors

The main purpose of the on-site visit is to observe the laboratory under routine conditions in order to check that it is operating in accordance with standards set down by the NTP/NRL in the manual for national tuberculosis laboratories. It is essential that the observations are broad in scope, covering administrative as well as technical aspects. On-site assessments should check that the laboratory is following the guidelines for QC (see SECTION 2.2, page 8). In some cases, the visit will have a specific purpose, for instance, to respond to a high frequency of false positives in a recent round of blinded rechecking.

A timetable for the evaluation of a peripheral laboratory might be as follows:

1) The intermediate level sets a date, decides on the scope of the visit, and nominates personnel to perform the evaluation.
2) The peripheral laboratory is informed of the date to ensure that relevant staff will be in attendance. (The laboratory must be given advance notice.)
3) The peripheral laboratory is informed of the scope of the evaluation and names of persons who will conduct the assessment.
4) The evaluation is carried out.
5) At the end of the evaluation, a written report is prepared and discussed with local staff. If required, the report should include suggestions for corrective actions. Where appropriate, a date for a follow-up visit can be advised.
6) A copy of the report is forwarded to the NTP manager.

Checklists

Standard checklists will help to ensure that assessments are carried out in a consistent and structured format. Checklists improve the efficiency of on-site evaluation. Sequential checklists give a reliable picture of the laboratory's performance over a

period of time. They can also assist the NTP in measuring country-wide laboratory performance, prioritizing resources for retraining, etc.

The EQA consensus document makes the following point:

> "… a simple checklist requires well established standards of acceptability and extensive training for consistent application and recording of what is observed to be unacceptable."

It is important to note that although the checklist will guide the supervisor in the assessment, information obtained must always be supplemented by general observations of the laboratory's operation.

Checklists to suit local conditions must be prepared by the NTP/NRL in accordance with guidelines from international authorities (e.g. WHO). Although checklists may vary between countries, there should be a common format to ensure that key areas of the laboratory's operation are assessed.

The NTP/NRL will need to develop two checklists for the two categories of supervisory visit. While the two checklists will be similar in structure, the one used by laboratory personnel will understandably be more detailed and emphasize technical issues.

(CHECKLISTS are discussed further in the following chapter.)

2.3.2 PANEL TESTING

Panel testing refers to the process by which the peripheral laboratory (known as the "test laboratory") performs acid-fast microscopy on a set of prepared slides received from the central laboratory (the "reference laboratory"). This exercise checks both the laboratory's staining procedure as well as the ability of the technician to recognize and quantitate any acid-fast bacillus present. (If practicable, the test laboratory should return the slides to the reference laboratory to allow checking of stain performance, etc.) The panel will usually consist of 5-10 unstained smears. In cases involving poor staining performance at a test site, an alternative approach is to include both stained and unstained smears (e.g. six unstained, four stained) so as to gauge proficiency in smear examination. The panel should consist of a range of positives, as well as at least one negative. The reference laboratory must provide feedback to the test laboratory, including scoring for accuracy of the results as well as suggestions as to the likely explanations for any errors. Review (and in some cases, restaining) of the returned smears can provide helpful information.

A major advantage of panel testing is that it can provide a rapid picture of the proficiency of many laboratories in a country (or region). Distribution of the same panel to different laboratories will identify sites most in need of improvement. For

laboratories that see only rare positives, a panel test has an added advantage in that it can provide a "refresher" of the appearance of a positive smear. There are, however, a number of disadvantages in panel testing, some of which are inherent in the exercise itself. Technicians are likely to make a special effort with the test slides and the results might not reflect true performance. Also, preparation of many sets of standard slides including low-count positives is a challenge for even the most competent laboratories. Finally, transport of slides by post or courier can be a problem and slide positivity may fall with ageing and during transport delays in hot and humid conditions.

(The EQA consensus document provides full information on the critical issues in panel testing; recommendations for scoring; and procedures for preparing sets of slides of predetermined positivity.)

Panel testing in countries in the Western Pacific Region with a high burden of tuberculosis

In countries in the Western Pacific Region (WPR) with a high burden of tuberculosis there are large numbers (in some cases, many hundreds) of peripheral laboratories. The task of preparing and distributing uniform (and blinded) panels will impose a major workload on the reference laboratory. Similarly, review and collation of results and preparation of useful feedback reports will call for many staff with appropriate skills. Thus, it is recommended that panel testing be given low priority in WPR countries with a high burden of tuberculosis. Panel testing may, however, have value for certain laboratories with a history of poor performance, new staff, etc. It might also be appropriate for countries where there is only sketchy information on laboratory proficiency, and there is need for a rapid assessment in order to prioritize training and supervisory activities. In such circumstances, it is recommended that the NTP/NRL obtain assistance from an external source (e.g. a WHO Collaborating Centre) in supplying the sets of slides and in reviewing results.

2.3.3 BLINDED RECHECKING

Blinded rechecking refers to the process by which a random selection of slides collected from the routine workload at a peripheral laboratory (the "test" laboratory) is re-examined at an intermediate or reference laboratory (the "controlling" laboratory). The purpose of the exercise is to allow a statistically valid assessment of the proficiency of the peripheral laboratory. Each round of slide checking must be followed by feedback in the form of a written report, showing details of incorrect scorings and offering suggestions for quality improvement (corrective actions).

The EQA consensus document makes the following statement:

> "…blinded rechecking is the only EQA method that provides reliable assurance that a country has an effective AFB microscopy laboratory network supporting DOTS. All programmes should strive to implement a blinded rechecking programme."

A major advantage of blinded rechecking is that the controlling laboratory can check not only the scoring of the smear, but also the performance of the stain, the size of the smear, and the quality of the specimen – all of which influence the reliability of the final result. Thus, blinded rechecking is more powerful than panel testing.

Blinded rechecking will have significant resource implications for higher-level (intermediate) laboratories which act as controllers for the peripheral laboratories.

A new approach to sampling slides for blinded rechecking

Traditional EQA by cross-checking (as recommended in earlier WHO publications) involved re-examination of all positives, plus 10% of negative smears selected at random. This approach creates huge workloads for controlling laboratories. Recently, in one country in the WPR, more than 300,000 slides were re-examined, of which 135,000 were positive smears. The number of slides re-examined represents 16% of the total examined in that year. For most developing countries, cross-checking using this method would be unsustainable.

The EQA consensus document acknowledges the significant workload imposed by the previous sampling system and proposes a simpler system known as the Lot Quality Assurance System (LQAS). The authors make the point that:

> "use of a rigorous statistical approach would require complex sampling considerations … for many reasons, a strict statistical method is not practical and sustainable for most countries."

Under the LQAS method, peripheral laboratories are no longer required to store positive and negative slides in different boxes; slides are stored sequentially by laboratory number regardless of positivity. The sample size depends on the positivity rate, the total number of negative slides processed each year, and the expected performance (sensitivity) compared to the controllers. Table 7 (page 17) is taken from the EQA consensus document (Table V.1: Recommended Annual Sample Sizes [page 42]) and shows the numbers of slides recommended for blinded rechecking at various workloads and slide positivity rates. The table has been compiled on the basis of a sensitivity of 80% and specificity of 100% (for negative slides); acceptance number for errors equal to zero; and confidence level of 95%.

Table 7 **Recommended annual sample sizes** (as per Table V.1, EQA consensus document)

	Slide positivity rate					
No. of negative slides per year	5%	**10%**	15%	20%	25%	30%
200	107	72	54	43	36	30
500	154	89	62	48	39	31
1000	180	96	66	49	40	33
5000	208	**103**	69	50	40	33
50 000	216	104	69	51	40	33

If LQAS was applied in a country, where, for example, the national slide positivity rate is 10%, and where the average annual workload for peripheral laboratories is around 2800 negative slides, only 103 slides per laboratory would need to be rechecked during the year (see percentages in **bold**). As a result, the annual reduction in the number of slides re-examined by provincial laboratories each year would be around 80%. It should be noted, however, that this formula allows for zero errors, i.e., any detected errors over one year must be followed up. As stated in the EQA consensus document:

> "… if one or more errors are detected, the supervising laboratory must make subjective decisions as to whether these errors are random or represent a potential problem that requires investigation and, if needed, a subsequent intervention to improve performance."

In addition to bringing a major reduction in workload at the intermediate level, use of LQAS brings additional cost savings because fewer slides need to be transported from the periphery to higher levels. LQAS will also result in more efficient use of technical resources and skilled technicians can be redirected to other activities such as supervisory visits and training.

Slide selection by LQAS

The EQA consensus document contains detailed instructions for sampling slides by LQAS. See Appendix D (page 32) of this document for concise instructions and a practical example of slide selection.

Blinded rechecking and classification of errors

External quality assessment by rechecking relies on a blinded re-examination of the selected sample of slides at a higher-level laboratory. (See Appendix E [page 33] of this document for an outline of instructions for rechecking in the controlling laboratory.)

The technician performing the re-examination must have a high level of skill in acid-fast microscopy; have a thorough understanding of the sources of errors; and be trained in compiling the summary report that will eventually be returned to the peripheral laboratory (and NTP/NRL). It is essential that the technician performing the rechecking is at least as competent as the technician who issued the original result.

A discrepancy between the reported result and that found on re-examining is referred to as an "error". The EQA consensus document proposes a classification of errors based on correlation of results from the test and controlling laboratories. TABLE 8 is an adaptation of TABLE V.3: CLASSIFICATION OF ERRORS (page 49) in the EQA consensus document.

Table 8 **Classification of errors from results of slide rechecking**

	Result in controlling laboratory				
Result in test laboratory	**Negative**	**1-9 AFB/100 OIF**	**1+**	**2+**	**3+**
Negative	Agree	QE	**FN**	**FN**	**FN**
1-9 AFB/100 OIF	QE	Agree	QE	**QE**	**QE**
1+	**FP**	QE	Agree	QE	**QE**
2+	**FP**	**QE**	QE	Agree	QE
3+	**FP**	**QE**	**QE**	QE	Agree

QE = quantification error; FP = false positive; FN = false negative; OIF = oil immersion fields; **bold** = major errors
NB: A "false" result is defined by discordance between the reported result and the controlling laboratory's result.

It should be noted that the EQA consensus document uses a more complicated scheme for classifying errors. The simplified version in the table is adequate for making decisions as to the proficiency of the peripheral laboratories. As stated earlier, any error detected by LQAS must be viewed as a potential indicator of diminished competency, and investigated further. Repeated major errors (shown in **bold** in the table above) would almost certainly signal the need for prompt on-site assessment and/or re-training. An occasional minor error (quantification) is unlikely to be a signal of ongoing problems. The trend over time will be the best indicator of laboratory performance.

Whenever there is a discrepancy between the reported result and that found in the rechecking process, the peripheral laboratory must be informed as soon as is practicable. Furthermore, the controlling laboratory must give feedback that includes likely explanations for the discrepancy as well as suggestions for corrective actions. Technicians experienced in creative problem solving can be particularly helpful in explaining (and correcting) the major causes of incorrect results in acid-fast microscopy. Some common causes for errors detected by slide rechecking are shown in TABLE 9.

Table 9 **Common causes for "errors" in blinded slide rechecking**

Type of error	Possible causes	Suggested actions
False positive	■ artefact (e.g. stain deposits or crystals) incorrectly interpreted as AFB ■ AFB carried over in immersion oil from a previous positive smear ■ stained AFB have faded since original report	■ refresher course for technician ■ re-stain false positives and re-examine
False negative	■ insufficient time spent in scanning smear ■ incorrect microscopy technique ■ problems with staining (pale AFB, insufficient contrast in background) ■ defective microscope	■ refresher course for technician ■ prepare new staining reagents ■ on-site check of microscope performance
Quantification error (minor)	■ insufficient time spent in scanning smear ■ lack of understanding of scoring system	■ refresher course for technician
Quantification error (major)	■ lack of understanding of scoring system ■ incorrect microscopy technique ■ defective microscope	■ refresher course for technician ■ on-site check of microscope performance

3 Implementing quality assurance

3.1 Key issues

The consensus document on external quality assessment (EQA) makes the following statement:

> "Quality assurance (QA) of laboratory services is a complex issue highly dependent on resources in the country or region; structure of the health system and laboratory network; and incidence of disease."

It follows that in order to be effective, the laboratory QA system should be introduced in a gradual, stepwise process, and only after considerable planning and critical assessment of the strengths and weaknesses of the current situation.

Quality assurance of tuberculosis laboratory services will be unachievable (or unsustainable) without the following:

1) national commitment to the DOTS strategy (Directly Observed Treatment, Short-course);
2) adequate resources (personnel, operating budget, etc.) for DOTS;
3) a national policy on tuberculosis laboratory services;
4) a structured laboratory network closely integrated with National Tuberculosis Programme (NTP);
5) a high degree of competency and commitment at National Reference Laboratory (NRL);
6) adequate resources (trained personnel, budget, etc.) for all laboratories.

(It is presumed that all countries in the Western Pacific Region [WPR] with a high burden of tuberculosis already meet prerequisites 1, 2 and 3 above.)

In countries where DOTS is in a process of expansion, QA can be implemented in those regions where DOTS is already operating.

3.1.1 LABORATORY NETWORK

Quality assurance of laboratory services will be most effective when diagnostic laboratories are integrated with the NTP. In countries where tuberculosis is diagnosed in the private sector, efforts should be made to work collaboratively with such laboratories to ensure high standards of diagnosis at all levels.

The laboratory network will usually have a three-tiered structure as shown in TABLE 10.

Table 10 **Typical structure of laboratory network**

Level	Service area	Functions
Central	National (or regional)	■ national policy (methods, manual, QA) ■ training and technical support ■ QA planning and implementation ■ supervisory visits ■ equipment and procurement ■ research
Intermediate	Provincial	■ sputum smear microscopy ■ preparation, distribution of reagents ■ QA implementation ■ supervisory visits ■ training and technical support ■ data analysis
Peripheral	District, commune	■ sputum smear microscopy

As DOTS expands to cover the total population, there must be a parallel expansion of laboratory services. Ideally, a diagnostic laboratory should be located at each health centre providing DOTS services. Locations of DOTS centres (and laboratories) will be determined in part by factors such as demography, geography, access to transport, etc.

3.1.2 NATIONAL REFERENCE LABORATORY

The National Reference Laboratory (NRL) plays a key role in both delivery of diagnostic services at all levels, as well as in the planning and implementation of QA. It is therefore important that a competent laboratory is designated as the NRL. Such a laboratory will typically be associated with a large hospital or research institute and be located in the national capital. It is advantageous if the NRL is located adjacent to the NTP administration.

The NRL has the lead role in national tuberculosis control. For that reason, it must have the capacity required to oversee the development of the national network of

diagnostic centres. Senior staff should have appropriate training and experience, and have demonstrable commitment to high standards of scientific practice and laboratory management. Training and QA demand significant human and financial resources. Operational funding for the NRL should come from the NTP budget.

(In large, populous countries, there may be operational advantages in designating one or more r*egional* reference laboratories. Such laboratories must, however, work in close collaboration with the NRL.)

Operational costs of intermediate and peripheral laboratories will typically be funded by provincial health budgets. Competition for scarce resources can reduce the amount of funding available for tuberculosis laboratory services. It is essential that NTP managers work with provincial health authorities to ensure adequate support for local tuberculosis programmes.

3.2 Assessing the current situation

The EQA consensus document included guidelines for the steps that should be taken when implementing (or expanding) EQA in a particular country or region. It is recommended that the NTP/NRL in countries with a high burden of tuberculosis undertake a similar analysis (see TABLE 11 [page 24]).

(It should be noted that data on workloads, including slide positivity rates, are necessary for applying the Lot Quality Assurance System (LQAS) method for selecting slides for blinded rechecking.)

Quality assurance demands extensive and specific resources. During the planning phase, there must be a reasonably accurate estimate of the resources required – not only to commence QA, but also to ensure that it is sustainable. Quality assurance programmes derive their value from continued application. Therefore, if there is reasonable doubt as to continued availability of resources to support the QA programme, it should not be commenced.

The type and amount of resources required will be influenced by many factors, and will be different in every country. TABLE 12 (page 25) summarises the critical resources for the EQA tools recommended for countries in the Western Pacific Region.

Table 11 **Recommended steps for pre-implementation assessment**

Tasks	Key issues	Notes
1. Make a chart of the laboratory network, showing relationships and functions at different levels.	■ The network should be supervised by a central laboratory (NRL). ■ A laboratory network integrated with the NTP is the ultimate goal for effective tuberculosis control. ■ Laboratories at intermediate levels should support peripheral levels.	■ Where a formal network (NRL, etc.) is not yet established, as an interim measure, a provincial or regional laboratory may support QA in local peripheral laboratories.
2. Make an inventory of available resources (include staff, microscopes, budget).	■ Technicians should have received appropriate training for tuberculosis microscopy. ■ There must be an efficient system for ordering and delivery of supplies. ■ Each laboratory must have a suitable microscope (x 1000) in good working order. ■ The laboratory environments should be suitable for tuberculosis microscopy. ■ There should be effective communication between the laboratories and NTP. ■ Laboratories should have appropriate administrative support.	■ Microscope performance is critical to providing quality service. ■ Replacement of defective microscopes may not be necessary; some older microscopes can be serviced. ■ Electric binocular microscopes are recommended, although sputum smear microscopy can be performed by direct light microscopy. ■ If possible, there should be standardization of the type of microscope in use.
3. Collect data on specimen workload and assess adequacy of resources with respect to workload (include data on positivity rates).	■ Staffing levels should be sufficient to provide continuous service. ■ Approximate slide positivity rates (average, and range for all laboratories) are required for EQA by blinded rechecking.	■ Recommended maximum number of specimens/smears per technician per day is around 20. ■ Proficiency will be difficult to maintain in laboratories processing less than 500 samples annually (will depend on frequency of positives, also). ■ Laboratories with abnormally high or low workloads should be identified.
4. Document current QA activities. Collect data and evaluate performance. Identify limitations and causes of problems, lack of sustainability, etc.	■ Results of QA should be documented and forwarded to NTP/NRL (or provincial authority). ■ QA should lead to improved performance. Details of corrective actions should be documented. ■ District DOTS supervisors should be trained to evaluate basic laboratory operations.	■ Principles of QC should be part of training programmes. ■ QC should be part of everyday activities in all laboratories. ■ Informal QA and subjective assessments from programme personnel can provide useful information on laboratory performance.
5. Determine resources needed for implementing (or expanding) QA activities.	■ Eventual goal is for national QA programme incorporating on-site evaluation and blinded rechecking of slides (LQAS system).	

Table 12 **Critical resources for EQA**

	Critical resources		
EQA activity	**Personnel**	**Laboratory requisites**	**Other**
Supervisory visits	■ central-level (e.g. NRL) laboratory staff trained in all elements of QA for at least annual visits to intermediate laboratories ■ intermediate-level (e.g. provincial) laboratory staff trained in all elements of QA for at least quarterly visits to peripheral laboratories ■ DOTS supervisors trained in basic on-site evaluation of peripheral laboratories (use of checklist)	■ adequate numbers of intermediate level laboratories with capacity to support supervisory visits to peripheral level and to conduct retraining	■ funds to cover travel of staff from intermediate to peripheral level ■ checklist for supervisory visits by laboratory personnel ■ checklist for supervisory visit by non-laboratory personnel ■ standard quarterly data collection form for use by peripheral laboratories ■ system for delivery of reports to NTP
Blinded rechecking	■ intermediate-level laboratory staff with skills required for rechecking and evaluating Ziehl-Neelsen smears ■ DOTS supervisors trained in slide selection procedure by LQAS method	■ adequate numbers of intermediate-level laboratories with capacity to carry out slide examination, prepare feedback reports and conduct retraining ■ sufficient slide storage boxes to allow peripheral laboratories to keep all slides over at least one quarter	■ procedures for blind rechecking (including instructions for slide sampling by LQAS) ■ standard data forms that ensure "blinding" of the rechecking process ■ system for delivery of sampled slides to intermediate level for blinded rechecking ■ communication system to deliver feedback from intermediate to peripheral level ■ system for delivery of reports to NTP

3.3 Steps towards implementation

The EQA consensus document suggests the following steps, once the decision to implement QA has been made:

1) Plan specific steps to establish or improve EQA methods.
2) Define and obtain necessary resources.
3) Conduct pilot test and document results.
4) Evaluate and modify plans based on results of pilot test.
5) Expand EQA based on results of pilot test and resource availability.
6) Assess impact.
7) Modify or expand plan as needed.

There should be no need to conduct pilot studies as the effectiveness of the two recommended strategies has already been demonstrated. However, in most countries the most practicable approach will be to introduce EQA progressively. This is particularly so in those countries where QA is not in operation or has limited operation. Availability of resources at central and intermediate levels will determine the speed at which EQA can be implemented (or expanded). From the perspective of the NTP, the best results will come from introducing EQA in laboratories where deficiencies in service have already been identified.

Table 13 **Major topics for training in EQA**

Topic	Laboratory personnel	DOTS supervisors
Consequences of deficient laboratory service in DOTS	x	x
Basic principles of laboratory quality assurance	x	x
Sources of laboratory errors in tuberculosis microscopy	x	x
Critical elements of quality control in tuberculosis microscopy	x	x
Principles and procedures for on-site evaluation (simple)		x
Principles and procedures for on-site evaluation (detailed)	x	
Selection of slides for blinded rechecking	x	x
Procedure for blinded rechecking of slides	x	
Quality improvement (corrective actions) in tuberculosis microscopy	x	

3.4 Training of personnel

Once the decision to implement EQA in a particular region has been taken, it is essential that all personnel (laboratory technicians as well as DOTS managers) receive appropriate information and training. External quality assessment will not be effective unless all involved personnel have an understanding of its principles and practices. In the early stages of implementing EQA, it is recommended that personnel are selected with a view to their being used as resources for training other staff as EQA expands. The NTP/NRL must take a lead role in preparing documentation and providing financial support for training personnel. Major topics for training are shown in TABLE 13 (page 26). Note that the content of the training programme for laboratory personnel is different to that for DOTS supervisors.

3.5 Documentation

The NTP/NRL is responsible for preparation of relevant guidelines, checklists and data sheets that suit the local situation. Once prepared and trialled, the following items will ensure that quality assurance is implemented in a standard and effective manner:

- guidelines for quality control in laboratories;
- quarterly workload report for peripheral laboratories;
- guidelines for supervisory visits;
- checklist/report for supervisory visits (simple);
- checklist/report for supervisory visits (detailed);
- guidelines for selection of slides for blinded rechecking;
- data sheet for recording details of selected slides;
- guidelines for performing blinded rechecking;
- data sheet for reporting results of blinded rechecking.

(The above items should eventually be included in the manual for national tuberculosis laboratories.)

The EQA consensus document includes examples of both simple and detailed checklists for use in supervisory visits. Concise versions of these checklists and data sheets are included in the APPENDICES at the end of this document. It is recommended that these are used as templates for developing country specific checklists.

Appendices

APPENDIX A

Quarterly Workload Statistics

National Tuberculosis Control Programme

LABORATORY	
TECHNICIAN/S	
QUARTER	YEAR

REASON FOR EXAMINATION	PATIENTS			SPECIMENS		
	Number tested	Number positive	% positive	Number tested	Number positive	% positive
DIAGNOSIS						
FOLLOW-UP						
NOT-STATED						
TOTAL						

Number of specimens scored as *saliva*: ☐

Number of ZN control slides tested: ☐

Are ALL slides kept for checking: ☐

Are there any shortages of lab supplies: ☐

If YES, give details:

Any other comments:

Signed: .....

(Laboratory Technician) Date

[Copy to District DOTS Co-ordinator]

APPENDIX B

Blinded Rechecking Data Sheet

National Tuberculosis Control Programme

LABORATORY:	
TECHNICIAN/S:	
PERIOD OF REVIEW:	WORKLOAD:
DATE OF SAMPLING:	SUPERVISOR:

Sampling must not be done by the person who will re-examine the slides

	SAMPLING SEQUENCE	LABORATORY NUMBER	MICROSCOPY REPORT	COMMENTS
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				

Signed _____ *Signed* _____

(Laboratory Technician) Date (DOTS Supervisor) Date

COMPLETED DATA SHEET MUST BE KEPT SEPARATE FROM SAMPLED SLIDES

APPENDIX C

Blinded Rechecking Result Sheet

National Tuberculosis Control Programme

TEST LABORATORY:	
CONTROLLING LABORATORY:	
PERIOD OF REVIEW:	DATE RECHECKED:

	LABORATORY NUMBER	COMMENTS	CONTROL LAB RESULT	TEST LAB RESULT	CORRELATION
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					

TEST LAB	CONTROLLING LAB					
	NEG	1-9 AFB	1+	2+	3+	ALL
NEG						
1-9 AFB						
1+						
2+						
3+						
ALL						

SUMMARY

Major Errors (FN) =

Major Errors (FP) =

Major Errors (QE) =

Minor Errors (QE) =

TOTAL Errors =

Comments

Signed ..... *Signed* .....

(Laboratory Technician) Date (DOTS Supervisor) Date

[COPY TO TEST LABORATORY AND NTP/NRL]

APPENDIX D

Example of Slide Selection (LQAS Method)

National Tuberculosis Control Programme

The DOTS supervisor in Province ABC has made a visit to District XYZ on 10 October 2002. The supervisor will select a sample of slides from the 3rd Quarter, 2002 for blinded rechecking at the provincial laboratory.

- The slide positivity rate (SPR) for Province ABC in 2001 was 9%.
- The average number of negative slides examined at district laboratories during the year was 3 000.

From Table 7, the recommended number of slides for rechecking in one year is approximately 100 (i.e. 25 per quarter).

Thus, the supervisor will select 25 slides from the laboratory workload in Quarter 3.

To ensure blinding, the slide selection must not be performed by the district laboratory technician. Selection is performed by the DOTS supervisor, or alternatively, by a provincial technician, provided that a different technician will be performing the blinded checking.

1. Check the laboratory register and determine the number of smear examinations carried out in the previous quarter (e.g. 790).
2. Divide 790 by 25 (the number of slides to be selected) = 31.6. Round the figure down to 31.
3. In order to avoid any systematic sampling bias, the first slide number must be a random number. The number must be less than the sampling interval – in this case 31. For example, if the supervisor visited on the 13th of the month, circle every 31st slide starting from 13, i.e., 13, 44, 75, 106….757 (25 in total). (Alternatively, use the month of the year, or the first two digits on a bank note as a means of selecting the starting number.)
4. Go to the stored slides and locate the nominated slides. Ensure that the result is not showing on the slide. If a particular slide is missing or damaged, select the next slide. For example, if the 75th slide is missing choose the 76th slide.
5. Store the selected slides in sequence in a secure slide box.
6. Record full details of the selected slides on the BLINDED RECHECKING DATA SHEET. Ensure that all sections of the sheet are completed, including signatures of both the person who selected the slides and the local laboratory technician.
7. Record laboratory numbers of the slides on the BLINDED RECHECKING RESULT SHEET.
8. Transport the selected slides to the controlling laboratory and deliver to the technician along with the BLINDED RECHECKING RESULT SHEET.

APPENDIX E

Procedure for Blinded Slide Rechecking

National Tuberculosis Control Programme

The following procedure is performed by the controlling technician on receiving the slide box from the DOTS supervisor.

1. Check the contents of the slide box to ensure that all slides are present and that laboratory numbers match those on the BLINDED RECHECKING RESULT SHEET. (The slides should be filed in the sequence in which they were selected.)
2. Re-examine each smear as an "unknown" by the standard microscopy procedure. Record the result directly onto the result sheet and make note of other features such as specimen quality, smear size, staining intensity, presence of stain deposits under *comments*.
3. Deliver result sheet to the DOTS supervisor (or another senior independent officer) and transcribe the peripheral laboratory results from the BLINDED RECHECKING DATA SHEET.
4. Compare the results from the test laboratory with those from the controlling laboratory. (In cases of major discrepancies, another technician should be asked to verify the result. Where indicated, the controlling laboratory can re-stain any or all slides to assist in resolving major discrepancies.)
5. Complete the correlation table on the result sheet and make recommendations for corrective actions, if any.
6. Sign the BLINDED RECHECKING RESULT SHEET and make copies for the test laboratory and the local DOTS supervisor/National Tuberculosis Programme. File the original. (The slides can be discarded if there are no major errors. Slides with major errors should be shown to the technician at the peripheral laboratory.)

APPENDIX F

On-site Evaluation Report (short)

National Tuberculosis Control Programme

LABORATORY	SERVICE LEVEL
TECHNICIAN/S	
ASSESSOR	DATE

Section 1: ADMINISTRATION

SUB-SECTION	CHECKS	ASSESSOR'S COMMENTS
WORKPLACE	• dedicated work area • security, restricted access • efficient workflow • cleanliness, tidiness • water, electricity services	
STAFFING	• appropriate training for all staff • staff training records on file	
STANDARD OPERATING PROCEDURES	• method manual in laboratory • all staff know location of manual	
LABORATORY REGISTER	• register located in laboratory • register neat and legible • register up to date	
DATA COLLECTION	• quarterly workload report prepared • report is accurate	
EQUIPMENT	• maintenance records for equipment • manuals located with equipment	
SUPPLIES	• adequate laboratory supplies for next quarter • detail any recent delivery problems	
SAFETY	• protective clothing worn in laboratory • room has effective ventilation • fresh disinfectant readily available • staff have knowledge of TB symptoms	

Comments:

Section 2: SPECIMEN SUBMISSION

SUB-SECTION	CHECKS	ASSESSOR'S COMMENTS
COLLECTION	• training given to all relevant staff • instructions given to patients • collection in a ventilated area • appropriate containers in use • container labelled before collection • specimen quality checked pre-laboratory • completed request form with samples	
TRANSPORT	• efficient transport system in place • security maintained during transport • minimum delays in delivery	
HANDLING IN LABORATORY	• security maintained at all times • specimens processed within one day • patient details matched with request form • laboratory numbers written on side of jar • samples registered before processing • non-complying samples rejected • specimen quality assessed and recorded	

National Tuberculosis Control Programme

Comments:

Section 3: MICROSCOPY

SUB-SECTION	CHECKS	ASSESSOR'S COMMENTS
SMEAR PREPARATION	• smears prepared on new slides • smear labelled before smear prepared • smear approx 2cm x 1cm centered	
STAINING	• method agrees with NTP manual • all reagent bottles labeled • staining sink level • positive controls done at least monthly • results of controls recorded	
SMEAR EXAMINATION	• microscope is binocular, electric • microscope in good condition • microscope performance acceptable • spare bulb/s are available • check recent positive slides • scoring complies with NTP guidelines • at least 100 OIFs examined	
REPORTING	• all reports in standard format • new positives treated as urgent • all results reported as soon as practicable • all slides stored for rechecking	

Comments:

Have suggestions from previous round of slide checking been actioned? YES/NO
Give details:

SUMMARY AND RECOMMENDATIONS

Signed _____ ***Signed*** _____

(DOTS Supervisor) Date (Laboratory Technician) Date

[Copy to NRL/NTP]

APPENDIX G

On-site Evaluation Report (detailed)

National Tuberculosis Control Programme

LABORATORY	LEVEL
TECHNICIAN/S	
ASSESSOR	DATE

Section 1: ADMINISTRATION

SUB-SECTION	CHECKS	ASSESSOR'S COMMENTS
WORKPLACE	• dedicated work area • security, restricted access • efficient workflow • cleanliness, tidiness • water, electricity services	
STAFFING	• appropriate training for all staff • staff training records on file • staff adequate for workload • arrange retraining if required	
STANDARD OPERATING PROCEDURES	• method manual in laboratory • all staff know location of manual • methods reviewed at least annually • manual reflects current practice	
LABORATORY REGISTER	• register located in laboratory • register neat and legible • register up to date	
DATA COLLECTION	• quarterly workload report prepared • report is accurate	
EQUIPMENT	• maintenance records for equipment • manuals located with equipment • if balance used, check accuracy	
SUPPLIES	• adequate laboratory supplies for next quarter • detail any recent delivery problems • check for shortages of glassware, etc	
SAFETY	• protective clothing worn in laboratory • room has effective ventilation • fresh disinfectant readily available • staff have knowledge of TB symptoms • bio-safety cabinet providing adequate exhaust	
Comments:		

Section 2: SPECIMEN SUBMISSION

SUB-SECTION	CHECKS	ASSESSOR'S COMMENTS
COLLECTION	• training given to all relevant staff • instructions given to patients • collection in a ventilated area • appropriate containers in use • container labelled before collection • specimen quality checked pre-laboratory • completed request form with samples	
TRANSPORT	• efficient transport system in place • security maintained during transport • minimum delays in delivery	
HANDLING IN LABORATORY	• security maintained at all times • specimens processed within one day • patient details matched with request form • laboratory numbers written on side of jar	

National Tuberculosis Control Programme

	• samples always registered before processing • non-complying samples rejected • specimen quality assessed and recorded • specimen quality assessment is accurate	
Comments:		

Section 3: MICROSCOPY

SUB-SECTION	CHECKS	COMMENTS
SMEAR PREPARATION	• smears prepared on new slides • slide labelled before smear prepared • cross-contamination controls in place • smear approx 2cm x 1cm centered • smear is representative of specimen • drying and fixation as per method	
STAINING	• method agrees with NTP manual • all reagent bottles labelled • staining sink level • appropriate slide rack in use • positive controls done at least monthly • results of controls recorded • observe staining procedure • check heating time for carbol fuchsin • filter carbol fuchsin if precipitate forms	
SMEAR EXAMINATION	• microscope is binocular, electric • microscope in good general condition • microscope performance acceptable • microscope aligned correctly • spare bulbs are available • check recent positive slides • check thickness, cleanliness, counterstain • scoring complies with NTP guidelines • at least 100 OIFs examined • objective wiped after positive smear	
REPORTING	• all reports in standard format • new positives treated as urgent • results entered directly into register • all results reported as soon as practicable • all slides stored for rechecking	
Comments:		

Have suggestions from previous round of slide checking been actioned? YES/NO
Give details:

SUMMARY AND RECOMMENDATIONS

Signed _____ ***Signed*** _____

(Supervising Technician) Date (Laboratory Technician) Date

[Copy to NRL/NTP]