External Quality Assessment for AFB Smear Microscopy

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i. PREFACE

Effective control of tuberculosis (TB) is dependent on a network of local laboratories that provide accurate and reliable direct acid fast bacilli (AFB) microscopy testing for diagnosis, treatment, and monitoring. The availability and quality of AFB microscopy relies on national programs that support, train, and monitor the testing performance of individual laboratories. It is well known that serious deficiencies can occur in the laboratory operations when insufficient attention is given to the quality of the work product. The need to assess laboratory performance has been recognized for years and many National TB Programs have attempted at one time or another to monitor the quality of microscopy. Many countries, however, have no comprehensive laboratory external quality assessment (EQA) program or do not provide sufficient administrative support and attention. With the integration of AFB microscopy into general clinical services in many countries there is an increasing need to assure that the AFB smear is performed appropriately.

Workshops at IUATLD meetings (Bangkok-1998, Madrid-1999) have highlighted problems and new approaches for EQA of AFB microscopy at the country level. Participants at the 1999 workshop recommended that a practical guidance be developed to assist National Reference Laboratories in establishing (or implementing) and sustaining EQA programs for their local microscopy laboratories. With the support of IUATLD, WHO, JATA, and KNCV, the CDC and APHL have supported and coordinated a workgroup process to re-examine current EQA methods and develop a multi-sponsored international guidance document. The charge of this workgroup 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 were considered beyond the scope of the workgroup charge.

These guidelines describe several components of EQA programs. On-site evaluation of laboratories with standard checklists is a first step to promote effective and consistent supervision. Panel testing using sets of slides developed in the reference laboratory and administered to the peripheral laboratory is a mechanism that can be implemented with minimal resources. One priority is to develop consensus for standard protocols, logistics, and evaluation for the EQA method of rechecking a sample of patient slides from each local laboratory. The recommended approach is to use blinded rechecking of a sample of slides selected randomly from the laboratory register. The blanket approach of rechecking 100% of positives and 10% of negatives is not recommended since it is a burden for high-volume laboratories and inadequate for low-volume laboratories. In selecting sample sizes the workgroup focused on approaches that emphasize implementation and sustainability rather than rigorous analytical methods. The recommended rechecking sample sizes provide relative information on the sensitivity of microscopy within the microscopy network and is based on the annual laboratory volume of AFB smears and the proportion of positive smears. AFB positives slides are included primarily to achieve blinding, but the number is insufficient to determine specificity. AFB positive slides that are felt to be negative on rechecking (false

positives) are usually a systematic problem that can be readily detected and corrected. Programs are encouraged to use alternative approaches if false positives are an ongoing problem.

The workgroup, comprised of 14 members with experience and expertise in AFB smear microscopy, EQA, and TB control met on various occasions to develop and review draft documents and reach consensus. Consensus involves compromises on the different approaches promoted and used by many countries and organizations. Through co-sponsorship of a common approach in this guidance, the involved organizations have recognized the advantages of developing a single document to simplify the choices and promote adoption of some or all the EQA methods by each country NTP. Several drafts of this document were provided to the workgroup and invited experts. A draft was also reviewed by members and attendees during the 2001 IUATLD meeting. The final version went through review and clearance from all of the sponsoring organizations. In order to evaluate and improve the readability of the document, the final draft underwent a CDC sponsored formative evaluation with eight international consultants representing the target audience.

To improve the effectiveness of AFB microscopy networks, this document should be used by the NTPs and National Reference Laboratories (NRL) as a resource in developing country-specific guidelines. These international guidelines are intended as a comprehensive reference for method selection, implementation, and the many issues and interpretations that will be encountered in EQA programs. Implementing EQA will require each NTP/NRL to devote time and staff to first understand some complex technical and logistical issues and then select the methods that are most appropriate for the country. The co-sponsoring organizations recognize the challenge in developing simple country guidelines for EQA and therefore, are committed to supporting country-level implementation through additional training, technical assistance, and improving this technical guidance. This EQA guidance document is a first edition intended to educate and provide different approaches and perspectives on the critical issue of quality microscopy for diagnosis and monitoring. The biggest problem is not the technical differences among laboratory experts, but rather the lack of attention and resources given to microscopy networks in countries with a high burden of TB. In addition to providing guidance to National Reference Laboratories and NTPs, we hope that this focus on EQA for AFB smear microscopy will initiate discussion and research to refine recommendations based on country experiences.

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ii. GLOSSARY of TERMS

National Tuberculosis Program (NTP) Countrywide, permanent program responsible for activities directed at controlling tuberculosis through integrated efforts with the general health services for implementing the DOTS strategy promoted by WHO and the IUATLD.

DOT Directly Observed Treatment

DOTS The recommended strategy for TB control. This includes (1) government commitment to TB control activities, (2) case detection by sputum smear microscopy, (3) direct observed treatment (DOT) with standardized short-course chemotherapy, (4) a regular, uninterrupted supply of anti-TB drugs, and (5) a standardized recording and reporting system.

AFB Acid-fast bacilli

Peripheral Laboratory Laboratory located at primary health center or district hospital.

Intermediate Laboratory Regional or provincial laboratory existing in a larger hospital or city.

Central Laboratory May exist as part of the central public health laboratory or as an upgraded laboratory in the country's principal tuberculosis institution. Serves as the national reference laboratory for the tuberculosis program.

Reference Laboratory (RL) National reference laboratory or central laboratory. Plays an essential role in the organization and maintenance of the network of laboratories, and, among other things, develops guidelines for standardizing smear microscopy, assuring quality of testing, and overseeing training. Supports External Quality Assessment efforts in collaboration with the NTP.

District Used in this document to describe the administrative level at which the NTP is implemented. May be Region, Zone, Province, Governorate or Oblast.

Ziehl-Neelsen Stain (ZN) Acid-fast staining method using carbolfuchsin that is steam heated on the slides, decolorized, then counterstained with methylene blue. AFB appear red against a blue background.

Quality Assurance (QA) System designed to continuously improve the reliability and efficiency of laboratory services. Includes internal quality control, external quality assessment, and quality improvement.

> **Quality Control (QC)** Also called Internal Quality Assurance, includes all means by which the TB smear microscopy laboratory controls operation, including instrument checks and checking new lots of staining solutions.

External Quality Assessment (EQA) A process which allows participant laboratories to assess their capabilities by comparing their results with those in other laboratories in the network (intermediate and central laboratory) through panel testing and blinded rechecking. EQA also includes on-site evaluation of the laboratory to review quality of performance and should include on-site rereading of smears. EQA is an expansion of the proficiency testing as described by IUATLD.

Quality Improvement (QI) 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. Data collection, data analysis, and creative problem solving are the key components of this process. It involves continued monitoring, identifying defects, followed by remedial action including retraining when needed, to prevent recurrence of problems. QI often relies on effective on-site evaluation visits.

Proficiency Testing Historically, each organization has used this term differently.

(IUATLD) Assessment of laboratory capabilities by comparing results from different laboratories. EQA is an expansion of proficiency testing as defined by IUATLD.

(WHO) Process for sending smears from the reference laboratory to the peripheral sites.

(International Organization for Standardization ISO) Determination of laboratory testing performance by means of interlaboratory test comparisons.

Panel Testing Sending stained and/or unstained smears from the reference laboratory to the peripheral or intermediate laboratory to check proficiency in reading and reporting. Panel testing is equivalent to the WHO definition of proficiency testing. *The term panel testing is used in these guidelines in order to eliminate the confusion over the different definitions of proficiency testing.*

Rechecking Sending smears from the peripheral laboratory to a reference laboratory (intermediate or central laboratory) for rereading. These guidelines recommend that rechecking is always blinded, ensuring that the controller does not know the results from the peripheral laboratory. In other documents, this may also be referred to as rereading.

Controller Term used to describe the supervisory laboratory or technician responsible for rechecking slides.

Statistically valid sampling A method designed to obtain a random, representative subset of all slides which allows for quantitatively accurate conclusions.

Slide positivity rate (SPR) Proportion of positive slides among all those examined (diagnostic and monitoring) within a microscopy laboratory over a defined period of time.

Major error This type of error is considered the most critical since it has the highest potential impact on patient management, and can result in an incorrect diagnosis or

improper management of a patient. Major errors may indicate gross technical deficiencies, and include both High False Positive and High False Negative errors.

High False Positive (HFP) A negative smear that is misread as 1+ to 3+ positive[1]. This is a major error.

High False Negative (HFN) A 1+ to 3+ positive smear that is misread as negative. This is a major error.

Minor error In clinical practice, these errors may have some impact on patient management. However, for the purpose of evaluating laboratory performance, this type of error is considered less serious, because of inherent limitations in consistently detecting a few AFB that may be unequally distributed within a smear. The frequency of minor errors may indicate technical deficiencies.

Quantification Error (QE) Difference of more than one grade in reading a positive slide between examinee and controller. This is a minor error that generally has no impact on case management.

Low False Positive (LFP) Previously called a scanty false positive. A negative smear that is misread as a low (1-9AFB/100fields) positive. This type of minor error occurs occasionally even in laboratories that are performing well.

Low False Negative (LFN) Previously called a scanty false negative. A low (1-9AFB/100fields) positive smear that is misread as negative. This type of minor error occurs occasionally even in laboratories that are performing well.

Low Positive Term used in this document to describe 1-9 acid-fast bacilli per 100 fields, which is the WHO/IUATLD standard for quantitation. These results are reported to the physician as exact number of AFB seen. It is up to the physician and the NTP to decide if this represents a case or not. Previously referred to as a scanty positive.

Feedback Process of communicating results of EQA to the original laboratory, including suggestions for possible causes of errors and remedies.

[1] Based on IUATLD/WHO recommended grading of sputum smear microscopy results

I. INTRODUCTION

In many countries with a high prevalence of tuberculosis (TB), direct sputum smear microscopy remains the most cost effective tool for diagnosing patients with infectious tuberculosis and for monitoring their progress on treatment. The World Health Organization strategy for tuberculosis control (DOTS) relies on a network of laboratories that provide acid fast bacilli (AFB) sputum smear microscopy. The establishment of a broad network of well functioning peripheral laboratories within the context of the health system and readily accessible to the population is a high priority for any tuberculosis control program. If the laboratory diagnosis is unreliable, all other activities will be affected. However, the quality of laboratory services often may not be considered a high priority of the National Tuberculosis Program (NTP). Microscopy errors are likely to result in failure to detect persons with infectious TB who will then continue to spread infection in the community, or unnecessary treatment for "non-cases." Errors in reading follow up smears can result in patients being placed on prolonged treatment or re-treatment, or in treatment discontinued prematurely. Therefore, quality assurance of laboratory services, including AFB sputum smear microscopy, is essential. Both the availability and quality of AFB smear microscopy are dependent on national programs that support, train, and monitor the testing performance of individual laboratories.

This manual is intended to provide guidelines and methods to assess the quality and reliability of laboratory services. While these methods are not designed to review each and every patient diagnosis, the process of identifying and correcting problems in the laboratories will aid the NTP in efforts to assure overall quality of diagnostic services. Quality Assurance guidelines for all NTP services are beyond the scope of this document.

Quality Assurance (QA) is a system designed to continuously improve the reliability and efficiency of laboratory services. As defined by both the WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD), a quality assurance program for AFB smear microscopy has several components:

- **Quality Control (QC)** A systematic internal monitoring of working practices, technical procedures, equipment, and materials, including quality of stains.
- **External Quality Assessment (EQA)** A process to assess laboratory performance. EQA includes on-site evaluation of the laboratory to review QC and should include on-site rereading of smears. EQA also allows participant laboratories to assess their capabilities by comparing their results with those obtained in other laboratories in the network (intermediate and central laboratory) through panel testing and rechecking.
- **Quality Improvement (QI)** 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. Data collection, data analysis, and creative problem solving are the key components of this process. It involves continued monitoring, identifying defects, followed by remedial action including retraining when needed, to prevent recurrence of problems. QI often relies on effective on-site evaluation visits.

The National Tuberculosis Program and the National Tuberculosis Reference Laboratory (RL) have the responsibility to implement a Quality Assurance program for the peripheral and intermediate laboratories. In the absence of an established controlling authority, some level of quality assurance may be established through coordination and collaboration between the laboratory centers and the TB program. However, a successful QA program, including EQA and QI, cannot be fully implemented without support from the national or centralized reference laboratory. The NTP must, therefore, identify at least one laboratory that has the capability to serve as the National Reference Laboratory and provide the necessary resources to the reference laboratory and intermediate laboratories. Each country or program will need to evaluate the support structure and resources available in order to determine the most effective way to implement a quality assurance program.

Numerous technical resources for establishing TB laboratory services and performing direct AFB smear microscopy are available, including those developed by WHO and IUATLD. This document supports the technical guidelines and recommendations in these manuals, including requirements for internal quality control. Although broad, general guidelines for quality assurance of AFB smear microscopy are included in the technical manuals, there are many questions and controversies regarding External Quality Assessment. Other terms, including proficiency testing and external quality control are used to describe EQA in the various technical manuals. The definitions for these terms are not well standardized and can create confusion.

Therefore, this document is intended to provide more comprehensive guidelines for establishing or enhancing laboratory-based External Quality Assessment for the standard Ziehl-Neelsen (ZN) method for smear microscopy and for implementing remedial action to correct problems as part of overall Quality Improvement efforts. Although in some countries fluorescence smear microscopy is used in high-volume or reference laboratories, this manual does not address the additional complexities of EQA for fluorescent microscopy. As defined here, EQA is an expansion of proficiency testing as described by IUATLD. The EQA recommendations in this document are intended to replace (revise and update) the methods described in previous guidance from IUATLD and WHO. EQA includes:

- On-site evaluation of local TB microscopy services as well as inter-laboratory comparison of smear results through both panel testing and blinded rechecking. On-site evaluation includes regular visits by the district supervisor under the National or Regional TB Program, as well as an annual visit by a laboratory supervisor from a higher-level laboratory.

- Panel testing for evaluating performance by sending slides from the central laboratory to peripheral centers.
- Blinded rechecking to monitor performance by sending a sample of patient smears from the peripheral laboratories to a higher-level laboratory for rereading.

The guidelines presented here have been developed by a group of experts based on published literature as well as experiences in a number of countries with a variety of resource and infrastructure settings. They are intended as recommendations for the development and implementation of EQA for the majority of high prevalence, resource challenged countries, and therefore may not be applicable to all settings. Each country will need to determine the best way to use these recommendations. Descriptions of all of these methods, as well as general guidelines for use and implementation, are included in this manual. Detailed technical material, instructions, and forms for the different components of onsite evaluation, panel testing, and blinded rechecking are included as appendices, and may be useful to countries that wish to pursue modifications to the more general guidelines presented in the manual.

II. EQA: PLANNING & IMPLEMENTATION

Tuberculosis can be controlled successfully only in the context of a National Tuberculosis Program (NTP). The first priority of the NTP is case detection and cure by reliable diagnosis and effective treatment. Since case finding relies heavily on laboratory diagnosis, tuberculosis bacteriology is a fundamental component of a national TB control program, including successful implementation of DOTS. However, the laboratory services are often the most neglected component of these programs. Although quality assurance in tuberculosis laboratories is an essential component of effective tuberculosis control, quality assurance in the absence of an effective treatment program will have little impact and is a misplaced priority. Therefore, a well functioning national TB control program, including case finding by sputum smear microscopy and the delivery of effective treatment based on the DOTS strategy, is an absolute prerequisite to a successful Quality Assurance Program.

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. QA is a total system consisting of internal QC, assessment of performance using EQA methods, and continuous quality improvement of laboratory services. The ability to implement a quality assurance system will depend on the resources available and the stage of development of the NTP and laboratory network. This document has been developed to assist both the NTP and the national reference laboratory in establishing EQA for AFB smear microscopy that can be implemented and sustained with the resources of each country. Recognizing that the NTP may be in a gradual process of expansion, EQA should be implemented in areas or regions where DOTS is well established. In countries where health sector reform has been implemented, consideration should be given to integrating TB-EQA with other laboratory quality assurance programs such as those for HIV, STDs, and malaria.

Laboratory Network

It is important to provide TB smear microscopy services that are accessible to the entire population, yet maintain an acceptable level of technical proficiency. To accomplish this objective, a network of laboratories with competency in acid-fast sputum smear microscopy, supported by larger regional laboratories, and overseen by a National Tuberculosis Reference Laboratory, is required. This network of laboratory centers must have the capacity to plan and implement quality assurance activities in a well-organized fashion, capable of taking action to improve performance. Therefore the centers are typically organized according to the three typical levels of general health service:

Peripheral laboratories located at primary health centers or district hospitals. Staff have technical proficiency to perform sputum smear microscopy utilizing Ziehl-Neelsen (ZN) staining. Peripheral laboratories must be visited on a regular basis by a district supervisor,

who has been adequately trained to evaluate the basic functions of the microscopy laboratory.

Intermediate regional or provincial laboratories existing in larger hospitals or cities. Staff have technical proficiency to perform ZN microscopy, and may have capacity to perform fluorescence microscopy if volume is high. Intermediate laboratories should be capable of providing supervision, monitoring, training, and quality assurance to peripheral laboratories, including rechecking of smears.

Central may exist as part of the central public health laboratory, a research laboratory, or as an upgraded laboratory in the country's principal tuberculosis institution. Serves as the national reference laboratory for the TB program, with competence in direct ZN microscopy and, where appropriate, fluorescence microscopy. The national TB reference laboratory plays an essential role in the organization and maintenance of the network in terms of developing guidelines, ensuring high quality and standardized smear microscopy, and therefore must have the capacity to provide training and External Quality Assessment, including providing panel testing and rechecking to intermediate and peripheral laboratories.

EQA Method Considerations

As previously described, External Quality Assessment is one component of a laboratory QA program. **The focus of EQA is on the identification of laboratories where there may be serious problems resulting in poor performance, not on the identification of individual slide errors or the validation of individual patient diagnoses.** It is also an very important tool for communication with and motivation of laboratory technicians who may otherwise feel isolated in their work. There are three methods that can and should be combined to evaluate laboratory performance:

- On-site Evaluation
- Panel Testing
- Blinded Rechecking

Each method has distinct advantages and disadvantages (Table II.1), as well as varying levels of resource requirements. The choices for how to implement EQA in each country will depend on both the available resources and the ability to obtain additional resources to support the EQA activities. At its highest level, EQA includes a fully functional blinded rechecking program in addition to routine on-site supervision by trained laboratory staff. It is unlikely that any country will be able to fully implement all of the methods without a step-wise approach that takes into consideration the existing organizational structure, all of the available and projected resources, current knowledge of staff proficiency at the individual laboratories and the anticipated benefit to patient care. Early in the process, it may be useful to use EQA methods to demonstrate that performance problems exist in order to justify the additional resources needed to expand the activities and introduce improvement processes.

An important step in any process used to detect performance problems is the application of appropriate problem solving strategies. Many factors may contribute to poor performance, and training cannot be considered the universal solution. Therefore, resources to implement quality improvement are a critical consideration when designing a step-wise approach to EQA. Resources will also be necessary for ongoing performance assessment to evaluate the success of problem solving strategies. Developing an EQA process that is limited to the assessment of the current level of performance has little value unless the data is used to implement improvement strategies and measure ongoing performance improvement.

Table II.1 EQA Methods

Method	Advantages	Disadvantages	Uses
On-site Evaluation	• Direct personal contact • Motivating to staff • Observation of actual work • Identifies causes of errors • Permits verification of equipment quality and function	• Selective, usually not countrywide if left solely to the reference laboratory • Labor intensive • Costly	• Always during supervisory visits • Implement and monitor quality improvement measures • Data collection and flow of information among laboratory levels • Quarterly by district NTP supervisor • At least annually by the reference laboratory
Panel Testing	• Low workload for peripheral center • Improves laboratory credibility • Rapid response countrywide possible • Use of stained and unstained smears can help to identify source of problem • May lead to identification of faulty equipment	• Does not measure routine performance • High workload for central/reference laboratory • May not be motivating to improve daily performance	• Minimal first step for EQA with limited resources • Rapid assessment of gross deficiencies • Identify factors contributing to errors Assess training of microscopists
Blinded Rechecking	• Low workload for peripheral laboratory • Motivates improved daily performance • Reflects reality of routine performance	• Heavy workload for higher level center • Unavoidable inaccuracies • Biased if not blinded • Staff must be made available	• Countrywide • Standard for monitoring laboratory performance • Ongoing and permanent

On-Site Evaluation

Visits to the peripheral laboratories by trained laboratory personnel from the reference or intermediate laboratory are essential if performance is to be improved or maintained at a high standard. These visits allow for the observation of worker performance under actual conditions, including condition of equipment, laboratory safety, adequacy of supplies, and the process for smearing, staining, reading, recording and reporting. Stained smears can be reviewed during the visit. When problems are detected, solutions can be suggested and potentially implemented immediately.

DOTS requires a quarterly visit by a district supervisor. These visits provide an opportunity for basic supervision, including assessment of laboratory supplies, basic procedures and performance of internal QC. District supervisors should ensure that a functional microscope is available. In mature programs, non-laboratory supervisors may be trained to review a small sample of smears to detect any gross problems with smear preparation, staining and reading as well as function of the microscope. The supervisor can collect slides for rechecking, deliver slides for panel testing, or deliver results of panel testing and rechecking. A major advantage of on-site evaluation by properly trained personnel is the ability to identify sources of errors detected by panel testing or rechecking and to implement appropriate measures to resolve problems. Direct contact between the supervisor and the technicians motivates staff to improve performance.

When considering the resources necessary to implement on-site evaluation, the NTP will need to consider the:

a. Capacity of the reference laboratory staff to provide on-site evaluation of all intermediate laboratories at least annually.
b. Capacity of intermediate laboratories to provide on-site inspection of the peripheral laboratories at least annually, and more frequently as needed to correct problems identified.
c. Availability of properly trained non-laboratory personnel to make supervisory visits at least quarterly (as required for DOTS).
d. Capacity to implement necessary QI measures.

Panel Testing

A countrywide system for sending stained and/or unstained slides from the central laboratory to the peripheral sites for reading and interpretation at regular intervals is recommended as a minimum requirement to assess proficiency. This system may be established through initial pilot testing, with gradual expansion as additional resources become available. Panel testing is generally the least expensive and resource intensive of the three methods for EQA. However, this method only tests the technician's ability to stain and/or read smears, and is not a useful means to assess routine laboratory performance. Limited panel testing may be useful as a first measure of current performance when no other method for QA exists. Panel testing may also be useful in places where the intermediate laboratory structure necessary to support a rechecking program has not yet been established. The data obtained through a limited panel testing exercise can then be used to determine critical priorities for expanding EQA.

When considering the resources necessary to implement panel testing, the NTP will need to consider the:

a. Available financial support.
b. Proficiency of reference laboratory staff to perform ZN AFB smear microscopy.
c. Ability to demonstrate proficiency of reference laboratory staff through EQA, including panel testing.
d. Capacity of the reference laboratory staff to prepare panel testing slide sets for the laboratories to be evaluated
e. Available mechanisms to deliver slides to the peripheral sites, including mail and couriers.
f. Capacity of the reference laboratory staff to review and evaluate results from peripheral laboratories, and provide recommendations and follow-up for corrective action.

Blinded Rechecking

Blinded rechecking or rereading a sample of routine smears from the peripheral sites and intermediate labs by controllers at a higher level laboratory is considered the best method for evaluating performance and providing motivation to staff for improvement. **A countrywide program for blinded rechecking of slides at regular intervals should be the long-term goal for optimal EQA**. However, this method is the most resource intense and most expensive. Considerations to sample size and statistical validity will affect the required resources for a rechecking program. Using an appropriate statistical sample is most cost effective and efficient in high volume settings. Rechecking using the methods proposed in these guidelines for determining a statistically valid sample size will be less resource intensive for most high volume laboratories than sampling methods previously recommended. Rechecking using statistically valid sampling may not be feasible in low volume laboratories, low prevalence countries, or decentralized health systems. Planners need to balance resource constraints with statistical precision when determining appropriate sample size and sampling frequency for their program.

When considering the resources necessary to implement blinded rechecking, the NTP will need to consider the:

a. Available financial support.
b. Capacity of peripheral laboratories to store smears for rechecking.
c. Availability of properly trained personnel to collect appropriate samples of slides from peripheral sites.
d. Capacity of the reference laboratory staff at central and intermediate level laboratories to reread smears from peripheral sites, including second rereading to resolve discrepancies as needed.
e. Capacity of reference laboratories to provide results of rechecking as well as feedback to implement effective corrective action.

Process for Planning & Implementation

A systematic approach to developing and implementing EQA in a country or region should include the following steps. Assessment should be made using manuals published by WHO and IUATLD.

Step	Assess	Comments
1. Describe and diagram the laboratory network, including comprehensive list of all peripheral sites.	• Is there a formal laboratory network? • Is the network integrated with the National Laboratory and the NTP? • Do intermediate laboratories function in support of peripheral sites? • Does a centralized reference laboratory serve the network?	• As a starting point, the laboratory network may function on a regional or district level with intermediate laboratory serving as a reference resource for the region. EQA could be implemented on a regional level. • If laboratories are not integrated with the NTP, other agency or organization (e.g., NonGovernmental Organization NGO) may take responsibility for quality assurance.
2. Inventory available resources (actual and projected), including staffing, microscopes, supplies, and budget.	• Laboratory staffing at all level • Adequacy of supplies, and supply distribution • Function of microscopes • Effective communication channels • Appropriate administrate support (staff, forms, registers, computer systems) • Adequate financial resources	• Efforts should be directed at establishing a minimally acceptable level of microscopy service, including adequate numbers of properly trained technicians, replacement of bad microscopes, routine attention to minor repairs of lab equipment including microscopes, adequate supplies, mechanisms for communication, and program supervision. • Current and potential financial resources from both government and NGO sources should be assessed.

Step	Assess	Comments
3. Assess adequacy of current resources for current laboratory workload, positivity rates and infrastructure needs.	• What is the annual volume of slides in each microscopy unit? • Estimate the average and range of slide positivity rates. • Are there both high volume and very low volume peripheral laboratories? • Are data such as positivity rates available?	• Minimum volume of testing at peripheral sites should be sufficient to maintain proficiency in smear microscopy, but not so burdensome as to compromise quality. • Recommended volume per technician is at least 10-15 smears/ week, and no more than 20 smears/day • Laboratories processing <500 slides per year may not be able to maintain proficiency
4. Evaluate status and effectiveness of any current EQA activities. Assess reasons for current problems and limitations.	• What are the current activities? • What are the results of existing activities? • What are the strengths and weaknesses of existing activities? • Have there been any efforts to improve performance? • Are district supervisors trained to evaluate basic functions of microscopy laboratory?	• Even sub-optimal EQA activities may provide data on the current level of performance, helping to define the need for expanded activities with mechanisms for improvement. • Data from district supervisors may help to identify critical problems, including non-functional microscopes, inadequate supplies.

Step	Assess	Comments
5. Plan specific steps for establishment or improvement of EQA methods, including timetable for establishment of minimal, intermediate and optimal level activities.	• What are realistic short term and long term options for implementing or expanding EQA? • What methods fit best with the available resources? • Who are the important partners to include in the implementation and improvement process? • What is the priority for implementing each action step? • What is the timetable for implementing each action step?	• Consider the current level performance, if known, as well as any EQA activities currently in place. • In the initial stages of establishing EQA activities, very little information about performance will be known. Test panels may be the most efficient method to assess performance status. However, frequently repeating test panels may add little information. • Establishing a comprehensive countrywide rechecking program may take several years; therefore annual panel testing may be needed as an interim step. • In some areas with low incidence of tuberculosis or very few microscopy problems, a labor-intensive rechecking program may not be justified to detect only a few errors. Routine panel testing may be more cost effective.
6. Define and obtain necessary resources.	• Are additional resources available? • What are potential sources for obtaining additional staff, equipment, and microscopes, supplemental funds? • What is the timetable for obtaining new resources? • What data is needed to support the need for additional resources?	• Planning should attempt to minimize the gap between available and required resources. • Long term planning may be necessary to obtain adequate resources to fully implement EQA at the optimal level.

Step	Assess	Comments
7. Pilot test, document results.		
8. Evaluate and modify plan based on results of pilot.	• What implementation problems were discovered during the pilot test? • Can these problems be resolved prior to expanding EQA?	• Particular attention should be given to feasibility of workload, and to issues of validity of the controls
9. Expand EQA based on results of pilot tests and resource availability.	• What types of performance problems have been identified? • Is corrective action possible? • Are resources available to implement corrective action to improve performance? • What additional resources are necessary to expand EQA activities? •	• Planning may include intermediate steps, such as: o Limited panel testing o Countrywide or selective panel testing, followed by gradual implementation of rechecking o Gradual implementation & expansion of rechecking after pilot, without any panel testing.
10. Assess impact.	• Has corrective action resulted in improved performance?	• Improvement over time indicates that EQA methods are feasible and effective.
11. Modify or expand plan as needed.		

Resource Checklists

Resource requirements for each method of External Quality Assessment are listed below in order to assess both the currently available and the necessary resources when considering implementation or expansion of EQA processes.

1. On-site Evaluation
   - Reference laboratory staff to perform on-site evaluation visits for intermediate and peripheral laboratories annually. Consider availability of transportation.
   - Intermediate level laboratory staff to perform on-site evaluation visits to peripheral laboratories at least annually. Consider availability of transportation.
   - Properly trained supervisors (non-laboratory staff) capable of assessing basic operations in peripheral AFB smear microscopy laboratories at least quarterly.
   - Appropriate checklists to assess performance and operational conditions in laboratories.
   - Mechanism for implementing corrective action, including retraining if needed.
   - System to provide on-site evaluation results to the peripheral laboratory and back to the NTP or national reference laboratory on a timely basis.
2. Panel Testing
   - Procedures for preparing panel testing slide sets.
   - Reference laboratories capable of preparing test slide sets.
   - Adequate laboratory staff to prepare slide sets.
   - Functional microscopes at national, intermediate and peripheral laboratories.
   - Mechanism for distributing slide sets to peripheral sites without breakage or loss (mail, courier).
   - Adequate funds for sending slide sets to intermediate and peripheral laboratories and returning slide sets to central laboratory for review if necessary.
   - Staff for analyzing results.
   - Forms and communication system for reporting results back to program supervisors, test sites and technicians.
   - Process for corrective action and retraining if necessary.
   - Adequate funds to support retraining efforts.
3. Blinded Rechecking
   - Adequate number of laboratories and staff capable of rechecking slides.
   - Functional microscopes at national, intermediate and peripheral laboratories
   - System to determine sample size for rechecking.
   - Procedures for blinded rechecking process, including data analysis and resolution of discrepancies.
   - Infrastructure to support collection of slides including:
     - Properly trained staff to perform supervisory visits at 3 month intervals
     - Sufficient slide boxes for storage of all slides as defined by program
     - Mechanism and funds to deliver slide samples to higher level laboratory for rechecking.

  - Communication system for reporting results of rechecking back to program supervisors, microscopy sites and technicians.
- Process for corrective action and retraining if necessary.
- Adequate capacity to support corrective actions including funds and personnel to retrain supervisors and technicians as needed.

III. ON-SITE EVALUATION

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 program. Three different types of field visits can be used as part of an ongoing EQA process, depending on the resources available and the performance capability of the laboratory being visited.

- A monthly or quarterly visit to the laboratory by a district supervisor is required as part of the DOTS strategy for TB control.
- When very poor performance has been identified through panel testing or rechecking, an expanded visit by qualified laboratory personnel from a higher level laboratory (the intermediate laboratory or reference laboratory) may be necessary to perform a comprehensive evaluation of all laboratory procedures, implement corrective action, and provide training if needed.
- A routine visit by a laboratorian is recommended at least annually. Another option is to form quarterly supervision teams including intermediate lab staff and a district supervisor.

The NTP should use the WHO and IUATLD technical manuals and guidelines as the template to develop laboratory procedures and establish a system to monitor laboratory practices. The national laboratory must provide training to all personnel responsible for on-site evaluation. Non-laboratory personnel will need an adequate understanding of routine laboratory operations, including proper registration procedures, appropriate supplies, laboratory safety, basic microscope operations, and requirements of panel testing or rechecking programs operated by the NTP. Laboratory personnel must be knowledgeable in all operational and technical elements of AFB smear microscopy, and have sufficient expertise to observe technicians performing routine tasks. They should also facilitate quality improvement through on the spot problem solving and suggestions for corrective action when needed.

District Supervisor Visits

Monthly or quarterly visits to the health clinics by the district or regional supervisor are required as part of an overall DOTS program. In some countries with very limited resources at the National Reference Laboratory, or countries just beginning to develop an implementation plan for EQA, these visits may be the only type of on-site evaluation possible. On-site evaluation by non-laboratory personnel is generally limited to assuring that NTP requirements for recording and reporting of results are followed, and assessing operational conditions, such as safety, supplies, equipment and total workload unless these supervisors receive special training in laboratory issues. Supervisors should make sure that Standard Operating Procedures are in place, internal QC is performed, and a functional microscope is available. Since the ability to recognize AFB is considered essential for

anyone working in TB control programs where detection and follow-up are largely based on AFB-microscopy, some programs have had good experience using well trained district supervisors to read a few recent positive and negative smears as part of the routine quarterly visit. This decision should be made by each RL and NTP based on available resources and existing relationships between district supervisors and peripheral laboratories.

Visits by district supervisors are also useful to collect data on TB laboratory workload, positivity rate for suspects and follow up examinations. These data are often not available to the NTP, but are important for several reasons. Heavy workload (>20 smears per day per technician) may contribute to poor performance. A low workload (<15 smears per week per technician) may not be adequate to maintain proficiency in reading AFB smears. Workload for AFB microscopy may be more difficult to interpret in peripheral laboratories that perform a variety of laboratory tests. Monitoring slide positivity rates is necessary to determine appropriate sample sizes for a blinded rechecking program. Any significant changes in the indicators may indicate performance problems. For example, a change in positivity rate outside the expected range may signal a problem in over-reading or under-reading, especially if a new technician has been hired. Workload data and positivity rates are also useful to calculate necessary laboratory supplies.

Regular visits by the district supervisor also provide an opportunity to collect an appropriate sample of slides to forward to the higher-level laboratory for rechecking.

On-site Evaluation for Corrective Action

Extensive review of laboratory conditions and practices may be necessary when poor performance is identified during the quarterly supervisory visit, or through panel testing or rechecking, and the reasons for the performance problems are not readily apparent or are not corrected through more basic corrective action recommendations. On-site visits by experienced laboratory personnel from a higher-level laboratory provide an opportunity for immediate problem solving, corrective action and on-site retraining.

Regular On-site Evaluation by Trained Laboratory Personnel

Optimally, on-site evaluation should be performed at least once a year by personnel from a higher-level laboratory in order to evaluate the overall operational conditions in the microscopy centers. In many countries where health sector reform has been instituted, these visits should be integrated with evaluation of general health services and laboratory quality assurance activities for HIV, STDs and malaria. The annual (or more frequent, if needed) visit includes a comprehensive assessment of laboratory safety, conditions of equipment, adequacy of supplies as well as the technical components of AFB smear microscopy. Sufficient time must be allotted for the visit to include observation of all the work associated with AFB smear microscopy, including preparing smears, staining and reading of smears. On-site evaluation should also include examining a few stained positive and negative smears to observe the quality of smearing and staining as well as condition of the microscope.

Checklists

Every program will need to develop checklists to assist both laboratory and non-laboratory supervisors during the field visit and to allow for the collection and analysis of standard data for subsequent remedial action. Each country must establish a standard definition of what is acceptable for each checklist item, based on the guidelines established by WHO and IUATLD and the resources available in the area. An important component of using any checklist is to provide sufficient training and standardization so that the checklists are used consistently. Programs may refine the checklists to focus on problems that are frequently identified or most likely to occur, such as preparation of stains.

In addition to being sent to the NTP, results of checklists should always be sent back to the reference laboratory for analysis. A comprehensive list of all operational elements to be observed will help to ensure consistency in laboratory evaluations and provide immediate feedback to the technicians to facilitate rapid corrective action, as well as serve as documentation of the visit and record of current conditions and actions needed. An example of a comprehensive checklist for on-site evaluation is provided in Appendix A. This checklist contains open, non-leading questions and recommended observations along with objective criteria for acceptable practices. By using open, non-leading questions, as well as direct observation of the daily practices, the supervisor can assess how well the technician understands proper procedures, and is not just providing the expected "yes" response. This detailed checklist is provided as a template that may be adapted to meet the specific needs of EQA in each country. The preferred format should include simple, objective "Yes/No" evaluation criteria, yielding data that can easily be entered into a database for long term tracking and comparing performance.

A more simplified checklist, which may be more appropriate for use by well-trained district supervisors, is included in Appendix B. Use of a simple checklist can reduce the time necessary to evaluate a laboratory, especially when supervisors are very familiar with the process. Therefore, a simple checklist requires well established standards of acceptability and extensive training for consistent application and recording of what is observed to be unacceptable.

The on-site visit by both properly trained laboratory or non-laboratory personnel should make sure that:

1. Written standard operating procedures are available.
2. An adequate supply of reagents within expiration dates is available.
3. Proper, well functioning equipment and an adequate supply of consumables are available.
4. Internal QC is performed at the required intervals.
5. Laboratory safety practices are observed.
6. Record keeping is accurate and consistent with requirements of NTP.
7. Results are promptly reported to treatment centers or physicians.

8. A functional microscope is available. At a minimum, district supervisors must be familiar with simple microscope function, and be able to visualize a clear image through the microscope lens.
9. Patient slides are available and properly stored when EQA includes rechecking. Supervisors will collect an appropriate sample to be forwarded to reference laboratory.
10. Staff have received adequate training with refresher courses or corrective action are recommended when appropriate.
11. Workload and proportion of positive smears are evaluated.
12. Suspects recorded as smear positive in the laboratory register are recorded in the TB district register.
13. The findings and need for corrective action or additional resources are reported to the NTP.

On-site evaluation of the technical practices in the laboratory performed by properly trained laboratory staff from a higher-level laboratory includes all of the operational elements listed above, as well as:

1. Evaluating sputum collection procedures.
2. Observing and evaluating procedures for smear preparation, staining, and reading.
3. Assuring that positive and negative control slides are used with all newly made batches of stains as well as with each daily batch of smears.
4. Rechecking several positive and negative smears to evaluate staining, smear thickness, smear size, and results.
5. Reviewing results of panel testing and/or rechecking. Providing suggestions for corrective action or implementing corrective action as needed.

Documentation of any significant problems requires strategies and systems for improvement.

IV. PANEL TESTING

Panel testing is one method of External Quality Assessment that can be used to determine whether a laboratory technician can adequately perform AFB smear microscopy. This method tests individual performance, not the laboratory overall. Utilization of panel testing for EQA is considered to be less effective than rechecking because it does not monitor routine performance. Panel testing is useful to:

- supplement rechecking programs
- provide some preliminary data on peripheral laboratory capabilities prior to implementing a rechecking program
- assess current status of performance or to quickly detect problems associated with very poor performance
- evaluate proficiency of laboratory technicians following training
- monitor performance of individuals when adequate resources are not available to implement a rechecking program.

A panel consists of a batch of stained and/or unstained smears that are sent out by the reference laboratory to the peripheral laboratories for processing, reading, and reporting of results. Numerous issues must be considered for implementing panel testing, including:

- proper preparation of test smears
- number of slides to be included in the test panel set
- types of smears to include (stained and unstained, low positives, smears that are too thick or thin, poorly stained smears)
- mechanism for sending slides to the peripheral laboratories (post, courier, district supervisor)
- forms for test laboratories to record results
- time allowed for technicians in the test laboratories to complete panel and report results
- evaluation criteria for acceptable performance
- plan for reporting results to the test laboratory and implementing corrective action if needed
- mechanism to resolve discrepant results.

Preparation of Test Smears

There are several methods by which a set of panel testing smears may be prepared. The method chosen will depend on the resources available, and the current status of EQA in the country. Each method has significant advantages and disadvantages.

Prepared or Manufactured Smears

The reference laboratory may use known positive and negative patient specimens to produce a large collection of positive slides with a consistent, predetermined quantity of

AFB per slide as well as negative slides with authentic background material. By using manufactured slides, all laboratory technicians involved in the Panel Testing exercise will receive an identical set of slides, which should minimize variation in expected results due to variation in the consistency of smears. Well-manufactured slides with good consistency should result in demonstration of good performance by the technicians being evaluated. However, the process for preparing slides requires a high degree of technical proficiency, and a reference laboratory with appropriate equipment including a biosafety cabinet.

Two procedures for preparing panel testing smears are provided in Appendix C.1. The first procedure, which uses NaOH, has been validated in several countries. If the laboratory has repeated difficulties producing slide-to-slide consistency using the NaOH method, N-acetyl-L-cysteine (NALC) may be used as the mucolytic agent. The NALC procedure will be more expensive due to the reagent cost. Using NALC without NaOH may improve the quality of the smears; however, documented experience with this method is limited.

If manufactured slides are used for panel testing, every effort must be made to validate the consistency of slides prior to sending out test panels. This will ensure the reliability of panel testing results and document that reading errors do not represent a problem in the manufacturing process. Producing individual batches of slides with an identical number of AFB, especially low positives, requires practice to achieve slide-to-slide consistency. Each batch of slides must be validated by selecting a sample of >6 slides from each batch to be stained and read by different technicians to document consistency (Appendix C.2). To increase the efficiency of manufacturing slides, reference laboratories should develop the capacity to produce and validate batches of 50-100 slides as possible that can be stored for future use in preparing test panel sets.

Sending unstained slides for test panels has the advantage of testing several aspects of the microscopist's technical performance, including preparation of staining reagents, staining procedure, reading and reporting of results. Prepared AFB test slides can be stained by the reference laboratory prior to sending to the test sites. This will require much more effort on the part of the central laboratory in preparing test panels, but reduces the workload associated with panel testing for the laboratory technician being evaluated. Stained smears assess reading capability only, and do not provide any information on the technician‘s capabilities to prepare and stain smears. Requiring the technicians to report both the result as well as an assessment of the quality of the smear and stain may help the reference laboratory to determine the source of performance problems if technicians are unable to differentiate good smears from bad. Ideally, panel testing using prepared smears will include a combination of both stained and unstained slides. Results from this type of panel will help to identify if poor performance problems are due to the quality of the stain or staining procedure used at the peripheral laboratory or the actual reading of the smears.

Reusing Stained Patient Smears

When resources are extremely limited and technical expertise is insufficient to prepare smears, stained smear slides collected from the routine services at the reference laboratory may be used to develop test panel sets. Advantages of this method include low workload for the central laboratory, no requirements for special equipment, and the slide sets can be prepared quickly. However, this method tests only the ability of technicians to correctly read and report smears, not their capability to prepare staining reagents or properly stain smears. Another disadvantage to this process is the lack of consistency in panel sets. Each laboratory will receive an entirely different set of slides, which make it more difficult to correlate results between laboratories. For these reasons, slides with discrepant results will need to be referred back to the reference laboratory for review in order to ensure that the initial reading of the patient smear was correct, or that transporting the slides to the peripheral sites did not result in fading or degradation of the smear.

Number and Type of Smears

The number of slides to include in a set must be sufficient to make the exercise valid as a quality assessment indicator yet not add unnecessary burden to the workload of the technicians in the laboratory being evaluated. A limited number of slides, for example 10, which represents about half the maximum slides that a technician can examine per working day without losing quality, is an acceptable number.

The test panel must include slides with different grades of positivity in order to evaluate the ability of the technicians to properly grade positive slides. There is little value to including multiple 3+ smears since they present no challenge. It is important to send the same batch to all laboratories so that total performance of all participating laboratories can be evaluated. A panel testing exercise usually involves sending test panels with an identical composition (of negatives and positives) to many laboratories at the same time. So that technicians do not expect the same composition of slides each time, there must be variation in the slide sets (number of positives and negatives) sent with each new panel testing exercise. Although some countries have used the panel testing method as an opportunity to include "educational" challenges, such as smears that are too thick or poorly stained, there is no consensus on how beneficial this is in an overall EQA program.

A sample log sheet for tracking slides sets can be found in Appendix C.3. Some examples of an acceptable slide set, shown with increasing degree of difficulty:

1 slide graded 3+	1 slide graded 3+	1 slide graded 2-3+	1 slide graded 2-3+
1 slide graded 2+	1 slide graded 2+	2 slides graded 1+	2 slides graded 1+
1 slide graded 1+	2 slides graded 1+	3 slides graded 1-9 /100 fields	4 slides graded 1-9 /100 fields
2 slides graded 1-9 /100 fields	3 slides graded 1-9 /100 fields	4 negative slides	3 negative slides
5 negative slides	3 negative slides		

System for sending slides to the laboratories

The success of Panel Testing will rely on the ability to deliver slides to the peripheral laboratories with minimal breakage or degradation of the slides. If examinees receive packages of broken and faded smears, they will be poorly motivated to perform well, and confidence in EQA methods will decline. Each country will need to determine the best mechanism for delivering slides based on the services and resources available.

Options to consider include:

Mail/post should only be used in a country with a reliable postal system. It requires the use of suitable slide holders, such as plastic slide holders or heavy cardboard, to reduce breakage in transport.

Deliver during supervisory visits may be most effective in countries where regular visits by a district supervisor are well established. This should definitely be considered for delivering slides to laboratories that have demonstrated poor performance, as corrective action and quality improvement may be facilitated during the actual reading of the slides.

Courier System would be useful if a country has an established courier system in support of the NTP, health care system, or other activities.

Forms for Test Laboratories to record results

Standardized forms for recording and reporting results must be provided to the technicians in the peripheral laboratories. This will help to reduce confusion regarding the expectations and requirements of the exercise. Therefore, in laboratories with more than one technician, each technician responsible for routine testing must complete the test panel independently, and not as part of a group effort. It is important to instruct laboratory staff NOT to share results, since this is generally used as a method to evaluate the performance of individual technicians. Each technician must complete a form with his or her own results. A sample form that can be used by the technician to record results and by the reference laboratory to evaluate the results and provide feedback is included in Appendix C.4.

Time allowed for test laboratories to review panel and report results

Each program will need to set an appropriate timeline based on the conditions in the country. It is important that technicians be given sufficient time to read smears without significant impact to the routine workload. Technicians should spend the same amount of time reading test slides as they routinely spend on patient smears. Since technicians may spend an excessive amount of time reading slides when they know they are being tested, whenever possible supervisors should monitor the time spent reading panel smears. Reasonable turn around time is expected to be between one week and one month, depending on the delivery system, staffing and workload.

Frequency of testing

After initial pilot testing, panel testing should be done at regular intervals if it serves as the primary method for EQA. In the absence of a rechecking program, panel testing is recommended every 3-6 months, and no less than once per year. A reasonable interval should be determined based on resources available to distribute panels, evaluate results and implement corrective action. Panel Testing may also be done as a one time, initial exercise in the early stages of EQA to obtain baseline data on capabilities of laboratory personnel in the country. Panel testing may also be used intermittently as a supplement to rechecking.

Evaluation and Interpretation of Results

Panel testing evaluates performance using the best of smears, and generally the technicians know they are being tested. Therefore, we expect the best performance results when using this method. Standardized criteria for grading the results of each smear should be established. When designing a scoring system, both the number and the type of errors should be considered.

It is also helpful to determine the aggregate results from all laboratories before determining a final score. If a majority of technicians fail to report correct results for the same slide, it may represent a problem with slide preparation at the central laboratory, and results should be excluded from grading. A form for evaluating and reporting aggregate results is found in Appendix C.5.

Table IV.1: Classification of Errors

Result of technician	Result of Controller				
	Negative	1-9 AFB/100 f	1+	2+	3+
Negative	Correct	LFN	HFN	HFN	HFN
1-9 AFB/100 f	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	QE
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

Correct:	No errors	
QE	Quantification error	Minor error
LFN	Low False Negative	Minor error
LFP	Low False Positive	Minor error
HFN	High False Negative	Major error
HFP	High False Positive	Major error

Scoring System

A few different scoring systems are proposed here. It is important to consider the type of panel testing used when choosing a scoring system. A program that uses well manufactured slides can have a more rigid scoring system. New programs may want to design a scoring system that focuses on HFP and HFN. Mature programs should monitor minor errors more carefully. Each program will need to determine what is acceptable performance. The determination of acceptable performance (passing score) may be modified based on the first experience with panel testing and information about performance within the country.

1. Set of 10 slides, each slide is worth 10 points, total possible score = 100.
    a. Any positive called negative scores 0
    b. Any negative called positive scores 0
    c. Quantification error (2 grades) scores 5
    c. Passing score = 80
2. Set of 10 slides, each slide is worth 10 points, total possible score = 100.
    a. Each correct slide scores 10 points
    b. Each incorrect slide (any error) scores 0
    c. Passing score = 80
3. Set of 10 slides, each slide is worth 10 points, total possible score = 100.
    a. HFP and HFN scores 0
    b. LFP, LFN and QE scores 5
    c. Passing score = 80 – 90 (determined by NTP)
4. Set of 10 slides, each slide is worth 10 points, total possible score = 100.
    a. HFP and LFP scores 0
    b. HFN scores 0
    c. LFN and QE scores 5
    d. Passing score = 80

    (This scoring system may be used when there is need to focus on all false positives.)

An example of a report form is shown in Appendix C.4.

Feedback

Reports should include both individual results, as well as aggregate performance for all laboratories tested. Always send reports to the health authorities of the region/district, the local NTP supervisors/coordinators and the technician. Reports should include criteria for acceptable performance, possible sources of error and suggestions or requirements for remedial action. Sample forms for feedback are provided in Appendix C.4 and C.5.

Poor performance should always result in investigation to identify the reason. Investigation should include evaluating overall performance by all participating laboratories to determine

if the problem was poor slide preparation at the reference laboratory. For individual laboratories, investigation should include on-site evaluation to determine the source of the problem.

Technical supervisory visits offer the best opportunity to review results of panel testing with the technicians in the peripheral laboratories, identify potential sources of error, and implement corrective action. For this reason, on-site supervisory visits by experienced staff from the intermediate or national laboratory are recommended at least once a year, and more frequently if significant problems are identified.

All potential sources of error should be investigated, including quality of stains and staining procedure, quality of microscopes, and administrative procedures that may contribute to recording errors. All problems contributing to errors must be resolved. Possible causes of errors, and suggested evaluation steps are listed in Appendix E. Remedial training must be provided for technicians unable to properly identify AFB in smears. In some cases, no obvious problem will be detected.

When using the results of panel testing to demonstrate the need for additional resources, it will be necessary to evaluate the results of test panel performance as an aggregate of all laboratories tested. If a majority of laboratories submit unacceptable results, and it is determined that the consistency and quality of the slides used in the panel testing exercise was acceptable, this represents serious problems in AFB microscopy. Additional resources should be obtained for supervisory visits, correction of problems identified in individual laboratories, including replacement of microscopes (and/or microscope objectives), retraining if needed, and follow up panel testing. Panel testing may be used on a more limited basis if implementation of EQA by blinded rechecking has been broadly implemented.

Resolving Discrepancies

No system for developing test panels and distributing them to peripheral sites is completely without problems, which may include:

- Technical difficulties in preparing individual slides
- Error in the initial reading of a smear at the reference laboratory
- Incorrect recording of expected results
- Fading of stained smears during transport to peripheral sites

Therefore, any system for panel testing must include a mechanism to resolve discrepant results. This may require returning slides to the reference laboratory for rereading or sending a laboratorian from the reference laboratory to the peripheral site for comprehensive on-site evaluation and rereading of test panel slides with individual technicians.

V. BLINDED RECHECKING

Blinded rechecking is a process of rereading a sample of slides from a laboratory to assess whether that laboratory has an acceptable level of performance. Critical components of the accurate and practical rechecking system outlined in these guidelines include:

- the sample of slides from the laboratory should be a sufficient number of randomly selected slides to be representative of the performance
- the supervising laboratory, termed the controller, must blind the technician rechecking the slides from knowing the initial test results to prevent bias
- minor errors, representing false positive or false negative interpretations of 1-9 AFB/100 fields, are included with major errors for the purpose of obtaining a smaller sample size. The smaller sample size facilitates implementation and sustainability of rechecking programs
- discrepant results are resolved by a second controller
- there must be a system to provide continual feedback and improvements to the laboratories that are supervised

Strong and consistent support from the NTP is necessary to implement and sustain functional rechecking programs. This is the only EQA method that provides reliable assurance that a country has an effective AFB microscopy laboratory network supporting DOTS. All programs should strive to implement a blinded rechecking program.

Rechecking has been previously described in other manuals, including the technical guidelines published by the IUATLD. The rechecking method described here departs from previously published guidelines or established methods in several ways, including:

- Sampling 10% of negatives and 100% of positives is no longer recommended.
- Major and minor errors are included to achieving the smallest sample size.
- Positive and negative slides are no longer sorted or stored separately.
- Rechecking is always blinded, meaning the technician rereading the slide does not know the initial result.
- Discrepancies should be resolved by a second controller.
- Performance is assessed based on the number and type of errors exceeding a predetermined threshold, rather than calculating a percentage of errors.

Rechecking programs are intended to assess overall laboratory performance, **not** to confirm any individual patient's diagnosis. Therefore, the emphasis on rechecking every positive slide should be discontinued and replaced with a method that samples a representative collection of all slides—both positive and negative. If a laboratory has reported an unacceptable number of false positive results, which may be as few as one, this is most likely an indication of a systematic problem that can be detected by reviewing a sample and not all of the positive slides. The sampling method proposed in this chapter is

designed to sample the lowest number of slides that will provide an indication of whether a laboratory is meeting a predetermined performance goal. This method allows the is some statistical assurance that the laboratory is meeting performance expectations. **As with all current rechecking programs, 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 performance problem that requires investigation and, if needed, subsequent intervention to improve performance**. It is possible that after investigation in a particular laboratory, no serious problems will be found.

Although the concept of rechecking smears from the peripheral laboratories by a controller at a higher level seems simple, several important elements must be considered. A well functioning network of laboratories with an established relationship of collaboration is necessary. Rechecking requires a large investment of human and logistical resources. There must be sufficient number of staff at the intermediate and central laboratories to perform the rechecking. If controllers are overburdened with rechecking in addition to routine work, they may make more mistakes in reading than the peripheral labs. To determine the necessary resources, the national program must consider a system for all the necessary steps in a rechecking program:

1. Determine a valid sample size.
2. Properly store slides until sample collection.
3. Collect a random and representative sample from the laboratories.
4. Recheck smears, ensuring blinding.
5. Resolve discrepancies between original result and result of controller.
6. Interpret errors and establish corrective action requirements.
7. Report results of rechecking to the peripheral laboratory and to the NTP.
8. Investigate potential sources of errors during on-site evaluation.
9. Provide remedial training or other corrective measures.

Determining Sample Size

A major challenge in designing a rechecking program is ensuring that results reflect actual laboratory performance. Ideally, the collected smears should constitute a statistically representative and random sample based on both test volume in the laboratory being evaluated, and the expected performance parameters that must be defined by each country. However, if rechecking is to be feasible and reliable, workload for the controllers must also be considered.

The sample sizes presented here are based on statistical sampling methods. The use of a rigorous statistical approach, however, would require complex sampling considerations. For many reasons, a strict statistical method is not practical and sustainable for most countries. Therefore, a simple approach is presented, recognizing that implementing and sustaining a rechecking program outweighs the need for statistical precision. In this system,

sample size depends on the positivity rate, total number of negatives slides processed each year, and expected performance (sensitivity) compared to the controllers. This allows for the detection of laboratories where the number of errors exceeds the acceptable level that has been established by the NTP. A detailed explanation of the statistical methods and additional tables are provided in Appendix D.1 as further information for programs that may want to adjust sampling parameters.

Slide Positivity Rate (SPR) This is the proportion of positive smears among all slides (diagnostic and monitoring) in the laboratory from which the sample is Being taken. This number is estimated using the laboratory registers from the previous year or the preceding four quarters. Sample sizes can be set using the average positivity rate for a laboratory, region, or country.

SPR = (Number of positive smears per year/ Annual slide volume) x 100

Total Negative slides Annual slide volume minus the number Positive slides per year.

Sensitivity This is the expected performance in detecting positives, as compared to the controllers. Acceptable sensitivity should be determined by the NTP and NRL. The sensitivity, as defined here, is the detection of all positives, including low positives (1-9 AFB/100). Therefore, an overall sensitivity of 75-85% is recommended. New programs may want to start by using a sensitivity of 75-80% because this will reduce the sample size significantly, which may help to make implementing a rechecking program more feasible. Although a sensitivity of 75-80% may be perceived as too low by some NTP's, it is important to note that increasing the expected sensitivity will significantly increase the sample size for rechecking, making it difficult to implement or sustain rechecking. Even with a sensitivity of 80%, errors will still be detected in many laboratories. This does not automatically mean that the laboratory is not performing at the expected level; errors should be evaluated based on the type and frequency of occurrence. Additionally, some laboratories may find that they have a sensitivity higher than 80% once rechecking is implemented. Table V.1 is based on a sensitivity of 80%.

The number of slides to be selected (sample size) should be fixed beforehand by the program managers using Table V.1. Determining sample size should not be left to the supervisor collecting the slides or to the technicians. Ideally, one sample size can be chosen and used for all centers in the area as shown in Table V.2. If variation in slide volume or positivity rate among the centers in a supervisors' area is considered to be excessive, a few choices depending on the ranges of volume and positivity rate may be given. In areas with extreme variability, collectors might even be given a list with individual sample sizes per laboratory based on each laboratory's performance the previous year.

Table V.1 Recommended Annual Sample Sizes[1]

Slide Positivity Rate

Number of negative slides/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
50000	216	104	69	51	40	33

[1] Based on LQAS method applied to the negative slides with sensitivity of 80%, specificity of 100%, Acceptance number d=0, and 95% Confidence Interval. Each sample size was then increased proportional to the positivity rate to yield the final sample size that includes both positive and negative slides.

* Select the row with the number of slides/year closest to the district average volume or to the laboratory actual volume

Table V.2 Sample Size Determination Example

Procedure **Example**

Step 1

Make a list of the microscopy laboratories in your country (or region in large countries), with the following information:

- number of slides done per year
- number of positive slides per year
- number of negative slides per year

Laboratory	Slides/yr	Pos/yr	Neg/Yr
A	1 500	200	1 300
B	2 550	351	2 199
C	1 990	156	1 834
D	2 085	151	1 934
E	900	85	815
F	1 158	100	1 058
G	1 250	125	1 125
H	885	101	784
I	2 569	335	2 234
J	500	55	445
Total	**15 387**	**1 659**	**13 728**

Step 2

Calculate the slide positivity rate (SPR) in each laboratory and round off to the nearest %.

SPR = (Number of positive slides per year / annual slide volume) x 100

This is best done using Laboratory Register data from the previous year. Both diagnostic and follow-up slides should be included.

Laboratory	Slides/yr	Pos/yr	SPR
A	1 500	200	13%
B	2 550	351	14%
C	1 990	156	8%
D	2 085	151	7%
E	900	85	10%
F	1 158	100	9%
G	1 250	125	10%
H	885	101	11%
I	2 569	335	13%
J	500	55	11%

Procedure	Example
Step 3 Calculate the average SPR for your country (or region) and round off to the nearest % • Average SPR = (total positive slides / total number of slides) x 100	Average SPR = (1 659/15 387) x 100 = 10.8% **or** 10% (rounded off)

Note: If variation in slide volume or positivity rate among the centers in a supervisors' area is considered excessive, a few choices depending on the ranges of volume and positivity rate may be given. In areas with extreme variability, collectors might even be given a list with individual sample sizes per laboratory based on each laboratory's performance the previous year.

Step 4 Calculate the average annual number of negatives slides and round off to the nearest 1000 • average workload = number of slides done / number of laboratories	Average workload = 13 728 / 10 = 1 373 **or** 1 000 (rounded off)

Note: The sample size does not vary considerably when the annual workload exceeds 1000; therefore, rounding off will not affect the calculation.

Step 5 Decide on acceptable limits for performance in your country (or region). • Relative sensitivity (ability of technicians to detect AFB relative to the controllers) Recommended: 75% if new program 85% if established program	80% selected
• Acceptance number (maximum number of errors allowed before action is taken) Recommended: 0 if limited resources available 1 if adequate resources available	0 selected

Note: Because of the inherent limitations of AFB microscopy, 100% relative sensitivity is not possible. Agreement between technicians and controllers should be close to 95% for highly positive (2+/3+) smears, but may be as low as 30%-50% for low positives (1-9 AFB/100 fields). For this reason, a relative sensitivity based on reasonable expected overall performance should be selected.

Note: The acceptance number has a direct impact on the sample size - the larger the acceptance number, the larger the sample size required. To achieve the smallest, most efficient sample size, a value of 0 is recommended, but this means that a single error should be considered as a warning of possible problems and requires further evaluation. Increase of the acceptance number to d=1 will allow one error, but will result in a big increase in the sample size. The acceptance number is explained in more detail in Appendix D.1.

Note: Choosing 0 errors means that one can be 95% certain that a laboratory has met the performance goals if no error is reported. However, since both major and minor errors are included in calculating sample size, interpreting individual laboratory results should take into account both the number and the type of errors, as well as the trend over time.

Procedure	Example
Step 6	
Select appropriate sample size table.	Table V.1
Table V.1 can be used by most laboratories or regions if a sensitivity of 80% and acceptance number of 0 is chosen.	When choosing a different sensitivity or acceptance number, refer to the tables in Appendix D.3 and D.4.
On the left side of the Table, look down the first column to find the average workload of negative slides in your country/region per year.	Average number of negative slides = 1 000
At the top of the Table, identify the average SPR in your country/region, as calculated above.	Average SPR = 10%
Locate the corresponding sample size at this point	Sample size = 96

Procedure	Example
Step 7 Decide on a convenient interval to select the slides. • Recommended 4 x per year, i.e. Quarterly Divide the required sample size by the interval to calculate the number of slides to be collected at every interval.	96 / 4 = 24 slides to be collected every quarter

Step 8 Systematically collect the slides using the Laboratory Register Divide the number of slides processed during the interval (e.g. quarter) by the sample size. If a slide is missing, select the next slide in the Laboratory Register, irrespective of the result and continue systematically, using the sampling interval	Suppose 250 slides have been processed during the last quarter. 24 slides need to be collected, therefore: 250 / 24 = 10.4 Collect every 10th slide

Slides are collected from the entire sample of slides irrespective of whether the result was positive or negative. This method of random sampling will ensure that the number of positive, negative, false negative, and false positive slides in the sample is representative of the entire set of slides processed by the laboratory. This sampling system eliminates the need to select positive slides separately from negative slides; therefore, there is no need to store positive and negative slides separately. This also helps ensure blinding, since the whole sample will be naturally well mixed when the batch goes to the controller.

Collecting Slides (Sampling)

If the results of a rechecking program are to be a valid representation of routine laboratory performance, the sample collected must be random and representative of all the smears read by the technicians in the laboratory, and the results of the peripheral laboratory must be blinded to the controllers. The technical requirements for sampling are outlined here. Each national program will need to consider these requirements, establish a standardized plan and ensure that the proper resources for sample storage and collection are available.

Slide Storage

The laboratory must store slides in a way that allows retrieval of every slide identified for the rechecking sample. Therefore, it is best to save all slides, storing them in the slide boxes in the same order as they are listed in the laboratory register. In order to maintain consistency with the laboratory register, two blank spaces should be left behind the first slide from a suspect patient so that the second and third slides can be added after they are read.

It may be impractical for high volume laboratories to keep all slides; therefore, each program should determine an appropriate number based on the sample size needed and the frequency of sampling. A sufficient number of reusable slides boxes must be provided to save the required number of slides, using a system that involves discarding the slides in the oldest box and refilling with new slides. Low volume laboratories should have a sufficient number of boxes available to save all slides.

Slides must be labeled in a manner consistent with the laboratory register to ensure that the correct slide is matched to the result. The result of the smear examination must not appear on the slide.

Prior to placing slides in the storage boxes, slides may be cleaned with xylene to remove most of the immersion oil. If xylene is not available, excess oil should be allowed to drain off the slides. Store slides in boxes that allow the immersion oil to drip off, and the slides are not touching each other (e.g., do not stack or press slides together). Always store slides in closed boxes away from direct sunlight.

Slide Collection

Like most survey operations, rechecking requires motivated and well-trained staff to collect slides in order to ensure that a random sample is obtained. To avoid bias, the technician in the peripheral laboratory must never perform the sampling. In many countries, the supervisor will collect the sample during the quarterly visit. Some training and direction on how to sample from the laboratory register is critical. A less desirable alternative is to forward all slides and a copy of the laboratory registries to the intermediate or central laboratory.

Slide Selection

In order to eliminate selection bias, slides are selected using the laboratory register. This ensures that the technicians keep all slides, regardless of result or quality. Slides must not be selected from the slide box.

As shown in Table V.2, it is recommended that one quarter of the total sample size be collected during the quarterly supervisor visit. Slides are collected from the entire set of slides irrespective of whether the result was positive or negative. Following this approach, during the course of four quarterly collections (one year), a sufficient annual sample size will have been accumulated to allow for a statistically precise conclusion.

Once the supervisor identifies which slides are to be collected on the collection form, the technicians may collect the slides from the boxes. Technicians should be able to readily retrieve all of the slides. If a slide is missing, substitute the next slide as identified in the laboratory register, regardless of the result. Document the substitution on the collection form. If numerous slides are missing, this may indicate there is a problem in the laboratory. Problems may include that technicians may be destroying slides that were of poor quality, all slides are not being read, or technicians may not understand the need to save slides for rechecking. The supervisor should carefully consider the problem and provide criteria for corrective action.

Table V.2 Sampling Example

The average number of negative slides processed by the district laboratories is approximately 1000 smears per year, with a positivity rate of 10%. According to Table V.1, the annual sample size for blinded rechecking is 96 smears per year, so approximately 24 slides are to be collected during each quarterly visit. The supervisor calculates that the laboratory processed 250 slides since the last visit; therefore, every tenth (10^{th}) slide is collected to randomly obtain the required 24 slides.

Laboratory Register

Lab Serial Number	Date	Name	Sex M/F	Name of treatment Unit	Address New Patients	Reason for examination		Results of specimen			Signature	Remarks
						Diagnosis	Follow Up	1	2	3		
								Neg				
								Neg	Neg	Neg		
								Neg	Neg			
								Neg				
								5afb	Neg	(7afb)		
								Neg	Neg	Neg		
								Neg	Neg			
								Neg	Neg	Neg		
								Neg				
								(Neg)	Neg	Neg		
								Neg	Neg	Neg		
								Neg	Neg	Neg		
								Neg	(Neg)			
								Neg	Neg			
								Neg	Neg	Neg		
								Neg	Neg	Neg		
								3+	(2+)	2+		
								Neg				
								Neg	Neg	Neg		
								Neg	Neg	Neg		
								Neg	(Neg)			

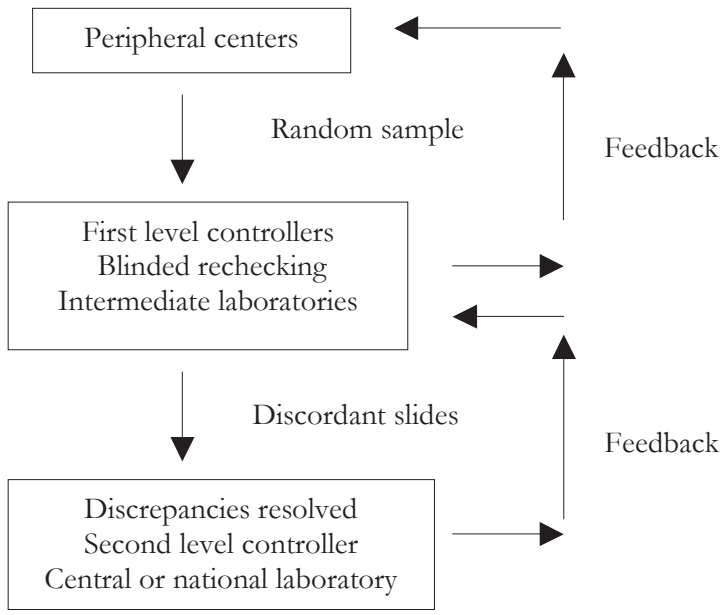
Rechecking Process

Reexamination must be done using the same technique as used in the peripheral laboratory to ensure that the technical characteristics of the method are comparable. The controllers must have demonstrated proficiency with the Ziehl-Neelsen staining method. The same number of fields as specified in the national guidelines for routine AFB smear microscopy should be examined by the controllers. The microscopes used by the controllers must be of good quality and in good condition.

Rechecking also provides an opportunity to assess related performance elements at the peripheral level. Smears may be evaluated for specimen quality (sputum vs. saliva), appropriate size and thickness, and quality of staining. Problems detected by the controller should be noted on the form, as this information may be very useful to supervisors responsible for providing feedback to the peripheral technicians, assessing possible reasons for high false positive or false negative results, and implementing plans for retraining and corrective action.

In AFB smear microscopy, absolute accuracy is impossible to achieve due to the absence of a reliable gold standard. Acid fast microscopy is a technique with inherent errors, even when performed by the most experienced and motivated technicians. In order to distribute the workload of a rechecking program evenly, first level control will usually be performed at an intermediate level. Even though the controller at the intermediate laboratory may have higher qualifications than the technician at the peripheral first level, **it cannot automatically be assumed that the rechecking result is correct.**

Organization of Rechecking Process:



Rechecking must be blinded to ensure objectivity. The first controller rechecking the slide must not know the initial result. However, the second controller who is responsible for resolving discrepant results will need to search long enough to find any AFB or to reliably exclude the presence of AFB, so at this point it is helpful for both results to be known. This should be done in a way to make it impossible for the final controller to determine which result was from the peripheral technician and which was from the controller. When the second controller reviews more than 100 fields, this should be included in the report sent back to the peripheral laboratory to show why there was a discrepancy (ex: 5 AFB/ 300 fields).

Intermediate and central laboratories that serve as rechecking centers must also have their own performance evaluated. In other words, the person rechecking the slides should also have their work rechecked. Since the first controller is blinded to the initial result, evaluating their performance can be accomplished by using a second controller to resolve discrepancies. Feedback on the results of discordant slides, along with the slides, must be returned to the first controllers, and action taken to resolve any performance problems identified.

Types of Errors

Once again, it is important to emphasize that rechecking is not a method for validating individual patient diagnosis, but rather of assessing overall laboratory performance, detecting unacceptable levels of errors so that corrective action can be taken, and providing continuous motivation for good performance. For the purposes of EQA, the types of errors are classified on the basis of expected laboratory performance, not on the potential impact of patient management.

Table V.3: Classification of Errors

Result being rechecked	Result of Controller				
	Negative	1-9 AFB/100 f	1+	2+	3+
Negative	Correct	LFN	HFN	HFN	HFN
1-9 AFB/100 f	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	QE
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

Correct:	No errors	
QE	Quantification error	Minor error
LFN	Low False Negative	Minor error
LFP	Low False Positive	Minor error
HFN	High False Negative	Major error
HFP	High False Positive	Major error

Discrepant Results

Discrepancies between the initial result and the results of the controller should be resolved by a second controller. Without this, it is impossible to identify the source of the error, and there is a risk of mistakenly informing the peripheral microscopists of errors. The discrepancies may be resolved in the central laboratory, other intermediate laboratory, or by a supervisor in the same laboratory. For the purpose of EQA, the result of the second controller is considered "final," and establishes whether the error was made at the peripheral or first controller level. Even with reasonably good performance at the peripheral and intermediate laboratories, it is reasonable to expect that 5-10% of smears in the rechecking sample will need to be reexamined by a second controller in order to resolve discrepancies.

While total absence of discordant slides from a larger collection (several centers) strongly suggests that rechecking was in fact not blinded, and is invalid. In fact, results from a rechecking scheme should be continuously analyzed for their validity, by comparing error rates (total FN, LFP) and numbers of HFP committed by first controllers on one hand and the total of their centers on the other hand. Controllers should have clearly less FN and almost no HFP for the controls to be valid. If controllers have clearly higher FN rates than their centers, the FN rates for the centers are certainly also under-estimated. In case centers are proficient and both first and second controls are well done, LFP will be equally divided between the peripheral centers and the first controllers. Unequal distribution of LFP (and sometimes also HFP) may indicate a problem at one of the controlling levels.

It has already been noted that acid-fast microscopy is a technique with inherent limitations. In addition to the fact that some discrepancies in reading AFB smears are to be expected, several technical problems have been described that may influence rechecking results. Although the actual impact of these potential problems remains controversial, it may be important for individual countries to consider these factors when organizing a rechecking program and interpreting results.

Fading

It has been well established that fuchsin stain is unstable in direct sunlight and in conditions of high humidity with high temperatures. The amount of time it takes for complete fading depends on several factors, including consistency of the smear and clumping of the AFB and the quality of the staining process. Excessive fading may contribute to an excessively high number of false positives detected during rechecking. Restaining may be necessary to resolve these discrepancies.

Staining Problems

Restaining may also be helpful in resolving problems with high false positive results that may be due to inadequate decolorization, stain precipitates or other problems with smear preparation and staining process. In some cases, AFB may be washed off fixed smears

during restaining; however, this usually occurs only with thin smears from liquefied or concentrated sputum. In specimens with very low numbers of AFB, this may result in a report of false positive by the controller.

Poor quality stain or problems with the staining method at the peripheral laboratory are important causes of false negative results. The classic recommendation for rechecking is to read smears in the condition in which they are received so that staining quality can be evaluated. However, problems with staining that result in unstained AFB may not be readily apparent to controllers, and important causes of error will remain undetected. For this reason, restaining of all smears prior to rechecking has been recommended by some authors. This may considerably increase the workload associated with a rechecking program, does not allow for a judgment of the staining quality, and remains controversial. Further research on the utility and benefits of restaining all slides is needed

Interpretation

When establishing a rechecking program, it will be important for the NTP to establish standards for acceptable performance, as well as recommended investigation steps and appropriate actions to correct problems. This system for rechecking is designed to look at both the number and the type of errors found when evaluating laboratory performance. Even though the sample sizes listed in Table V.1 are based on a sensitivity of 80% compared to controllers, it is still likely that one or more errors will be found even in laboratories that are performing at or above the expected level. This is an important concept for the National Reference Laboratory and the NTP to recognize when providing feedback to the peripheral laboratories. Logically, a rechecking program will start by focusing on major errors and on laboratories with large numbers of errors. When first starting a rechecking program, it may be necessary to assess current level of performance through limited rechecking to determine what performance level will trigger further action once the program is established.

If there are no errors, the performance goal has been met. If errors are detected, the interpretation and appropriate action may be different depending on the number and type of error, as well as the resources and capacity of the program.

High numbers of false positives should be a very rare occurrence. An isolated HFP is often due to a clerical error or poor record keeping at the peripheral laboratory. An error in sampling, where the wrong slide is collected, can cause occasional false positives. Slides initially reported as 1+ to 3+ positive that are repeatedly found to be negative by the controllers may be due to improper registration, deliberate cheating, grossly inadequate technique, poor quality microscopes, or simply total neglect. Higher rates of HFP are typically due to unusable microscopes or untrained or inexperienced microscopists, especially in centers with a low number of sputum smear examinations. If almost all of

the positive slides are HFP, accompanied by numerous HFN, the cause is most likely due to an unusable microscope. Since virtually any HFP result is an indication of a problem, there must be prompt investigation and implementation of any required corrective action.

An occasional HFN is to be expected due to inherent problems in the technique. Higher rates are often seen when technologists are overworked, and additional staff may be necessary to resolve the problem. False negatives may also be due to technical problems such as poor stains, insufficient staining time or heating, bad microscopes, or inadequate training. As with false positives, high number of false negatives may indicate gross neglect and an overall lack of motivation.

Low false positive and low false negative errors are to be expected, again due to the inherent problems with AFB smear microscopy. Low positive is defined by the IUATLD and WHO as 1-9/AFB per 100 fields, and such results do occur regularly[2]. As AFB are not homogeneously distributed in sputum, very few may be detected in an examination of 100 fields by one technician, but another technician examining a different 100 fields may not be able to find them. For these reasons, interpretation of low false positive and low false negative errors may be considered separately from major HFP/HFN errors.

Although LFN and LFP errors are minor (due to inherent limitations of the test), it is important to include them in designing a rechecking program because these types of errors constitute a more sensitive indicator of performance. Larger numbers of minor errors may represent performance problems in the peripheral laboratory, and it may be useful to address these issues once gross deficiencies have been resolved. Once major problems are resolved, minor errors also serve as on ongoing monitor of performance and as a means to validate the rechecking results since you would expect to see a similar rate of these types of errors from both the peripheral technicians and controllers if overall performance is equivalent.

Regularly finding more than just a few low false positives along with occasional high false positives may indicate that the technician is not completely clear about the recognition of AFB, and additional training may be needed. A high frequency of low false negatives may indicate a problem with heavy workload resulting in superficial microscopy. Poor quality microscopes or insufficient light may also contribute to high numbers of low false negatives.

Quantification errors (QE) are of minor importance in the initial implementation phases of EQA. Considerable variation in quantification is usual, only because of the reading of

[2] The term scanty is not used in this document because it has been used interchangeably to describe both 1-3 AFB/100 fields and the currently WHO/IUATLD recommended category of 1-9 AFB/100 fields. In the ATS-scale, most often used in low, but also in some high prevalence countries, scanty is defined as less than 1 AFB per 100 fields. The latter result is quite rare, and does not correlate well with culture results (ref. Kubica G P. Correlation of acid-fast staining methods with culture results for mycobacteria. Bull Int Union Tuberc 1980; 55: 117-124). In countries where the ATS scale is applied, scanty false negative errors as well as rare scanty false positive errors might even be ignored.

different fields by different controllers. For this reason, quantification errors are defined as difference of at least two grades when reading positive slides. However, correct quantification can at times be helpful to the clinician for decision making in difficult cases, so it is an ideal one could gradually be strived for. Besides, consistent under-reading of numbers of AFB can give useful indications in the investigation of high false negative error-rates.

Suggested examples of different interpretation methods:

a. No errors of any type is considered a target for optimal performance. Any major error (HFP or HFN) is unacceptable performance and triggers corrective action. Minor errors would be reported back to the laboratory, but the laboratory performance is still considered acceptable unless they continue to appear in more significant numbers.

b. No errors of any type is considered a target for optimal performance. Any major error (HFP or HFN) may indicate unacceptable performance and should trigger an evaluation and corrective action if needed. It is possible that no significant problems in laboratory practice will be found, and performance trends should be monitored over time. Minor errors require further evaluation only if they exceed some predetermined number, or exceed the average number seen in all centers in the program, or if the number of minor errors over time demonstrates a trend.

c. No errors of any type is considered a target for optimal performance. Any HFP and more than three LFN is unacceptable performance and triggers corrective action. One or two HFN may indicate unacceptable performance and should trigger an evaluation and corrective action if needed. It is possible that no significant problems in laboratory practice will be found, and performance trends should be monitored over time. Minor errors require further evaluation only if they exceed some predetermined number, or exceed the average number seen in all centers in the program, or if the number of minor errors over time demonstrates a trend.

Feedback

The primary purpose of a rechecking program is to improve the overall quality of smear microscopy, therefore regular and timely feedback to the peripheral laboratory is essential if any improvements in performance are expected. Annual reports should be sent to the regional health authority, district physician as well as the laboratory technicians. Although final analysis of the results and conclusions have to await completion of rechecking of the whole (annual) sample, preliminary observations, feed-back and remedial action will often be possible at the end of each sampling period. This will be obvious in laboratories with very poor performance where immediate problem solving is most urgently needed.

If results from the controllers are to be perceived as credible, and offer an opportunity to

improve performance, feedback should include returning slides with discordant results to be reread by the original technicians. This gives them a chance to show what they interpreted as AFB, or to be shown AFB they have missed.

Poor performance should always be investigated to identify the reason. The investigation should include on-site evaluation visit to determine the source of the problem. In most programs, the district supervisor will bring the rechecking results to the peripheral laboratory during the routine visit, which provides an opportunity to discuss results, recognize good performance and find potential solutions to any problems.

Visits by the supervising laboratory offer the best opportunity to review results of rechecking with the technicians in the peripheral laboratories, identify potential sources of error, and implement corrective action. For this reason, on-site supervisory visits by experienced staff from the intermediate or national laboratory are recommended at least once a year, and more frequently if significant problems are identified.

All potential sources of error should be considered, including quality of stains and staining procedure, quality of microscopes, and administrative procedures that may contribute to recording errors. All problems contributing to errors must be resolved. Possible causes of errors and suggested evaluation steps are listed in Appendix E. Remedial training must be provided for technicians unable to properly identify AFB in smears. In some cases, no obvious problem will be detected. Supplemental panel testing and ongoing blinded rechecking are recommended to monitor performance.

Due to the many variables that can affect laboratory performance, and the potential for these factors to change over time, it is recommended that rechecking be continued even after consistently good performance is achieved.

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VII. APPENDICES

Appendix A. Comprehensive Checklist

Appendix B. Short Checklist

Appendix C. Panel Testing

Appendix D. Blinded Rechecking

Appendix E. Investigation Error

A1: On-Site Evaluation Comprehensive Checklist

Laboratory: _____

District or Administrative Unit: _____

Laboratory Supervisor/Head of Laboratory: _____

Date of Visit: _____

Number of Microscopists/Technicians: _____

Current Laboratory Staff Qualifications: _____

_____

SECTIONS 1-9, and 14 MAY BE FILLED OUT BY LABORATORY OR NON-LABORATORY STAFF SUPERVISING THE CLINIC OPERATIONS. SECTIONS 10-13 ARE DETAILED LABORATORY EVALUATIONS THAT SHOULD ONLY BE COMPLETED BY TRAINED LABORATORY STAFF.

1. Standard Operating Procedures

Are written standard operating procedures for laboratory methods and equipment (e.g. NTP laboratory manual) available and accessible? Y N

If no, explain: _____

2. Laboratory Reagents

Observe and Question	**Indicator**			
Are all staining reagents available?	Reagent	Available	Within expiration date	Adequate Supply*
Have there been any shortages of reagents within the last three months? (*Adequate supply is defined as available current supply and no shortages over the past three months.)	Carbol Fuchsin	Y N	Y N	Y N
	Methylene Blue	Y N	Y N	Y N
Observe that all reagents in use are within expiration date	Sulphuric Acid 25% Or Acid Alcohol 3%	Y N	Y N	Y N
	Immersion Oil	Y N	Y N	Y N
Observe that Immersion Oil has acceptable viscosity (not too thick or too thin) (Will require training of non-lab supervisor)	Xylene	Y N	Y N	Y N

Explain any problems or deficiencies

_____

_____

_____

Action Required _____

3. Laboratory Supplies

Observe and Question	Indicator			
	Material	Available	Good Condition	Adequate Supply *
Are the following items available? Is the type of sputum containers in use approved by the NTP? Check to determine that slide boxes are adequate design (slides are stored standing up to drain oil and without touching each other) and number (sufficient boxes to store the number of slides required for adequate sampling) Have there been any shortages of supplies within the past three months? (*Adequate supply is defined as available current supply and no shortages over the past three months.) A clean water supply should be available distilled water is recommended. Water should be stored in bottles free of environmental contaminants including bacteria and fungus. Water from stagnant containers should not be used.	Slides Frosted Slides	Y N		Y N
	Slide Boxes	Y N	Y N	Y N
	Sputum Containers approved by NTP	Y N		Y N
	Diamond Pencil (or) Pencils (use with frosted slides)	Y N Y N	Y N	 Y N
	Wire Loops or Sticks	Y N Y N	Y N	Y N Y N
	Funnel	Y N	Y N	
	Filter Paper	Y N		Y N
	Staining Racks	Y N	Y N	Y N
	Spirit Lamp Or Bunsen Burner	Y N	Y N	
	Fuel for spirit lamp Or Gas for burner	Y N		Y N
	Lens Tissue	Y N		Y N
	Red Pen for recording Positive Results	Y N		
	Water supply	Y N	Y N	Y N
	Balance (for weighing reagents)	Y N	Y N	

Explain any problems or deficiencies

Action Required

4. Laboratory Safety

Observe and Question	Indicator		
Where is TB work performed?	TB work is performed in an area separate from other laboratory procedures	Y	N
	There are separate tables for smear preparation and microscopy	Y	N
Does the laboratory have adequate ventilation? If smears are performed in front of an open window, are technicians aware of air flow direction and potential for danger?	There is adequate & safe ventilation	Y	N
Which disinfectant is used?	An NTP approved disinfectant active against TB is used	Y	N
Have there been any shortages of disinfectant supply in the past three months?	An adequate supply of disinfectant is available	Y	N
How often are work areas cleaned with disinfectant?	Work areas are cleaned at least daily	Y	N
How are wire loops cleaned?	A sand bucket with Lysol or 70% ethanol is used to clean wire loops prior to flaming	Y	N
How are used slides disposed of? Are slides ever reused?	Used slides are properly disposed of (boiled or buried) If slides are reused, the are properly disinfected and cleaned, and never reused for AFB microscopy.	Y	N
How are used sputum containers disposed of? Are sputum containers ever reused? (Supervisor should check waste disposal site to ensure proper burial)	Sputum containers used only one time. Used containers are burned or properly buried.	Y Y	N N
Observe biohazard waste bin	A biohazard waste bin with a lid is available	Y	N
Are workers wearing lab coats?	Lab coats are worn while working in the laboratory	Y	N
Are lab coats removed prior to leaving the laboratory?	Lab coats are not worn outside the laboratory	Y	N
Are gloves used in the laboratory? Are they used properly?	If gloves are available, they are used in accordance with safe work practice recommendations	Y	N
Do workers wash their hands after working with sputum?	Proper handwashing procedures are followed	Y	N
Does laboratory appear clean and in good working order?	Lab is clean, layout is adequate to ensure safe practices	Y	N

Explain any problems or deficiencies

_____

_____

Action Required _____

5. Laboratory Request Form, Laboratory Register, Laboratory Reports

Observe and Question	Indicator	
Are the NTP approved laboratory request forms used for every patient?	NTP approved laboratory request forms are used for every patient	Y N
Are laboratory request forms submitted with complete information?	Laboratory request forms are submitted with complete information	Y N
Is the laboratory register present, and all columns completed properly?	Laboratory register is present Laboratory register is properly complete and legible	Y N Y N
Are patient records in laboratory register consistent with District Register? (Compare 10 patients from the Laboratory Registry and determine if all 10 patients are listed in the district register)	District TB cases appear in Laboratory register If no, how many patients are missing? _____	Y N
When is result information entered into the laboratory register?	Results entered into register daily	Y N
Are laboratory results recorded on the request form?	Laboratory results are recorded directly onto the form	Y N
How soon are results reported to the treatment center or physician?	Forms are sent back to the treatment center or physician within two working days. *All three results are sent back within two working days*	Y N Y N
Are three specimens routinely examined as recommended by IUATLD?	Three specimens, including spot, morning and spot are examined for diagnosis of TB.	Y N

Explain any problems or deficiencies

_____

_____

_____

Action Required _____

6. Microscope

Observe and Question	Standard	
Is microscope present? Adequate number of microscopes available?	At least one functional microscope is available Sufficient number of microscopes is available to manage workload	Y N Y N
Is the microscope functioning properly?	Supervisor can observe a clear image when looking through the microscope at a random smear.	Y N
Is the stage mechanism functioning?	Stage can be moved properly	Y N
Is adequate light source present?	Functional light bulb and electricity, or microscope is located near adequate light source	Y N
How is maintenance on the microscope performed?	Microscope is under maintenance contract or there is evidence of routine maintenance.	Y N

Explain any problems or deficiencies

_____

_____

Action Required _____

7. Storage of slides for External Quality Assessment

Observe and Question	Standard	
Are ALL slides kept as required by the NTP EQA program?	Slides are kept for EQA, supervisor is able to retrieve all slides identified from the laboratory register for EQA.	Y N
Are slides kept in storage boxes?	Slides are kept in storage boxes	Y N
Are slides cleaned with xylene before storage, or are slides stored in boxes so that oil can drain without contaminating other slides?	Slides are cleaned with xylene before storage, or are stored in boxes so that oil can drain without touching or contaminating other slides?	Y N

Explain any problems or deficiencies

_____

_____

Action Required _____

8. Staff Training

Has there been any change in staff since last supervisory visit?	Y N
Has new staff received proper training, as required by the NTP?	Y N
If training requirements are not defined by NTP, has each staff member participated in refresher training within past two years?	Y N
Have results of rechecking been received by peripheral lab?	Y N
Have results of rechecking or panel testing been acceptable?	Y N
If no, have any problems been identified through Rechecking or Panel Testing indicating there is a need for additional training/refresher course?	Y N

Explain any problems or deficiencies

_____

_____

Action Required _____

9. Workload

Number of smears last quarter		Number of suspects smears last quarter	Number of follow up smears last quarter	
Total:		Total:	Total:	
# Pos:	# Neg:		# Pos:	# Neg:

Average number of smears read by each technician per day? _____

THE FOLLOWING EVALUATION QUESTIONS SHOULD ONLY BE COMPLETED BY SUPERVISORY LABORATORY STAFF

10. Collection of Sputum Samples

Observe and Question	**Standard**	
Is lab technician responsible for collecting specimens?	If yes, complete all questions in this section If no, skip to section 11	Y N
Ask the technician to describe the instructions for producing sputum that are given to patient	Patients receive adequate instruction to produce sputum rather than saliva	Y N
Is the quality of specimen checked?	Specimen is evaluated visually for presence of sputum	Y N
When the patient produces saliva, is a repeat specimen collected?	Smears are not prepared from specimens recognized as saliva. Repeat specimens are requested.	Y N Y N
How many pre-treatment specimens are routinely collected for diagnosis? How many specimens are routinely collected for treatment follow-up?	Three specimens are routinely collected, following IUATLD and WHO guidelines for Spot, Morning & Spot collection.	Y N

Explain any problems or deficiencies

_____

_____

Action Required _____

11. Smearing and Staining Procedures

Observe and Question	Standard	
Does technician verify that container is properly labeled?	Containers are labeled with the health center code and the patient identification on the side of the container, not on the lid.	Y N
Are new slides used for sputum AFB smears? Are slides cleaned prior to use?	New slides are used for AFB microscopy. Slides are cleaned prior to use if greasy.	Y N Y N
How are slides labeled?	Slides are labeled with laboratory code, serial number and sequence identifier.	Y N
How often is Carbol Fuchsin filtered?	Carbol Fuchsin is always filtered before use	Y N
How often is Methylene Blue filtered?	Methylene Blue is filtered at least once a month or more often if precipitate is noted in smears	Y N
Is the wire loop cleaned in sand and sterilized by flaming after every smear? **or** Is a new wooden stick used to prepare each smear?	The wire loop is sterilized by flaming after every smear OR A new wooden stick is used to prepare each smear	Y N Y N
Are smears air dried completely prior to fixing?	Smears are completely *air dried* prior to fixing	Y N
Are slides properly heat fixed?	Slides are heat fixed by passing 3-5 times through flame	Y N
How many slides are usually stained in a batch?	*A maximum of* 10-12 specimens are processed at one time	Y N
What is the staining procedure used by the technician? How long are slides stained with CF and MB? How are slides decolorized?	Slides are stained with hot, steaming CF for 5 minutes Stain is not permitted to dry on the slide Slides are decolorized for 3 minutes, repeat decolorization is performed only when needed, slides are not over-decolorized Slides are counterstained with MB for 1 minute	Y N Y N Y N Y N Y N
How often are microscope lenses cleaned with lens tissue?	Microscope objective is wiped with lens tissue after every slide examination	Y N
How many fields are examined to report a negative smear?	The microscopist takes at least 5 minutes *and* examine 100 fields	Y N
How many fields are examined to report a positive smear?	An adequate number of fields is examined to provide accurate quantitation. For high positives, this may be 20-50 fields, for low positives, 100 fields should be read.	Y N
How are results reported?	Results are consistent with *NTP* recommendations for grading and reporting	Y N
Are known positive and negative smears included as an internal control? Observe availability of sufficient quantity of control slides.	Control smears are included.	Daily Each new batch of stain Never

Explain any problems or deficiencies _____

12. Onsite Rechecking

Laboratory supervisor should re-read at least three positive and negative smears during the on-site visit.

	Slide No.	Result Peripheral Lab	Result Supervisor	Staining AFB	Staining Background	Sputum or Saliva	Thickness and size of smear
+							
+							
+							
-							
-							
-							

Observations:	
Were results of supervisor consistent with laboratory result? Explain any problems:	Y N
Is staining of AFB and background acceptable? Explain any problems?	Y N
Does background material represent sputum? Explain any problems?	Y N
Are smears of proper thickness? Explain any problems?	Y N
Are smears of proper size? Explain any problems?	Y N

13. Rechecking and/or Panel Testing

Have results of rechecking or panel testing been acceptable according to performance expectations set by NTP? Y N

If no, have any problems been identified through Rechecking or Panel Testing indicating there is a need for corrective action? Y N

Explain any need for corrective action _____

_____

_____

14. On-Site Evaluation Summary

List any MAJOR problems identified during the on-site visit:

A. Operational Problems:

B. Technical Problems:

15. Name of person completing On-Site Evaluation: _____

Signature: _____

16. Signature of Laboratory Supervisor: _____

B.1: On-Site Evaluation Short Checklist

Laboratory: _____

District/Administrative Unit: _____

Number of Microscopists/Technicians: _____

Qualifications of current staff: _____

Supervisor/Head of Laboratory: _____

Date of Visit: _____

Visiting Supervisor: _____

Item	Adequate/ Acceptable *		Problems Identified
SOP	**Y**	**N**	_____
Separate area for TB work	**Y**	**N**	_____
Separate tables for specimen Receipt/smear preparation/ Microscopy	**Y**	**N**	_____
Power supply	**Y**	**N**	_____
Running water supply	**Y**	**N**	_____
Waste containers with lid	**Y**	**N**	_____
Waste disposal by Autoclave/burning/buried	**Y**	**N**	_____
Balance			
Adequate Stock & Supply of:			
Specimen cups	**Y**	**N**	_____
Slides	**Y**	**N**	_____
Stains	**Y**	**N**	_____

* NTP will need to establish standards for acceptance using IUATLD/WHO recommendations for equipment, reagents, and safety as well as national recommendations based on resources. All supervisors should be trained prior to conducting on-site evaluation.

Item	Adequate/ Acceptable *		Problems Identified
Smearing/Staining Equipment	**Y**	**N**	_____
Slide boxes	**Y**	**N**	
Microscopes	**Y**	**N**	_____
Laboratory Register			
Laboratory Forms	**Y**	**N**	_____
Personnel	**Y**	**N**	_____
Training status	**Y**	**N**	_____
Safety Practices	**Y**	**N**	_____
General order/Cleanliness			
Timely reporting of results to clinicians	**Y**	**N**	_____

Is QC using positive and negative control slides performed as required by the NTP? ❑ Yes ❑ No

Are all slides kept as required by the NTP EQA Program? ❑ Yes ❑ No

Are slides properly stored in slide boxes? ❑ Yes ❑ No

Workload

Number of smears last quarter		Number of suspect last quarter	Number of follow up smears last quarter	
Total:		Total:	Total:	
# Pos	# Neg		# Pos	# Neg

Overall remarks: _____

_____

_____

Action Required: _____

_____

_____

Rechecking and/or Panel Testing Results (refer to feedback form)

Have any performance problems (based on criteria set by NTP) been identified through rechecking or panel testing? ❑ Yes ❑ No

If yes, explain any need for corrective action:

_____

_____

_____

Has corrective action been adequately implemented? ❑ Yes ❑ No

If no, explain:

_____

_____

_____

C1: Preparation of Panel Testing Slides with Known Contents

1. Introduction

This procedure is a self-explanatory laboratory method for producing multiple test slides from AFB positive and negative samples. Your laboratory staff should read and understand both the procedure and the testing protocols before developing test slides. This procedure has been reproduced/validated in state and national laboratories. If your laboratory has difficulty in producing slides that meet the requirements for consistency you should either: 1) review the procedure with special attention to the steps of heating and re-suspension; or 2) select patient specimens with less mucus. The sample development procedure requires materials that are routinely available in a national or regional reference laboratory in a low-income country. If your laboratory has continued difficulties with clumping of AFB that prevents slide to slide consistency, the use of N-acetyl-L cysteine (NALC) may improve the quality of the slides. Your laboratory should demonstrate proficiency in producing samples with a minimum of 25-30 slides that are consistent for negative and low numbers of AFB before proceeding to developing test slide sets.

NaOH method
(ref Dr. Nguyen Ngoc Lan, Pham Ngoc Thach Hospital, Ho Chi Minh City, Vietnam and Dr. Alex Sloutsky, Massachusetts Dept. Health)

2. Materials Required

Note: Processing should be performed in a Biological Safety Cabinet.
50 ml plastic screw cap tubes
40% Formaldehyde
4% NaOH
Vortex
Water bath at 55-60°C
Distilled water
Centrifuge
Slides

Positive specimen (fresh specimens, no more than 2 days old, are preferred)

Amount: 3 ml or more;
AFB load: >2+ AFB by Ziehl-Neelsen direct smear;
Color: White to light green; *blood-stained* specimens should be avoided;
Thickness: Watery (less mucous) specimens are preferred to increase consistency.

Negative specimen (fresh specimens, no more than 2 days old, are preferred)

Amount: 5 ml or more;
Color: white to green;
Thickness: Watery (less mucous) specimens are preferred to increase consistency

Note: An AFB negative specimen with 20 or more white blood cells per field is preferred.

3. Preparation of AFB Positive Stock

a. Place 3 ml of AFB positive specimen into a 50 ml screw cap plastic tube. If volume of the specimen is more than 3 ml, aliquot it into separate tubes.
b. Add 1 drop (approx. 50 µl) of 40% Formaldehyde per 1 ml of sputum, vortex well.
c. Incubate for 1 hour at room temperature (25- 30°C).
d. Add 1 ml of 4% NaOH (if the sputum is too thick, add up to 2 ml of NaOH solution so that the final concentration of NaOH is always 1-2%).
e. Vortex thoroughly for 4-5 min.
f. Add up to 20 ml of distilled water, mix well.
g. Incubate in a water bath for 30 min. at 55-60°C, mix occasionally by inverting the tube during incubation. If there is no water bath available, boil a beaker of water, cool to 90-95°C and place the tube in the beaker for 20-25 min. It is important to maintain the incubation temperature in the 55-90°C range.
h. Add distilled water to a total volume of 40 ml, mix by inversion.
i. Centrifuge @ 3,000 x g for 20 min. at room temperature (25-30°C).
j. Decant supernatant carefully, add 0.5-1 ml of distilled water to resuspend pellets. If initial sputum was aliquoted into portions, pellets from the same specimen are combined, prior to resuspending.

Note: It is advisable to avoid specimens containing impurities (food remains etc.) However if the impurities are still found in the sediment after it is dissolved in distilled water, filter the specimen through the gauze and recentrifuge it.

4. Preparation of AFB Negative Stock

a. Distribute 3-4 ml aliquots of AFB-negative sputum into 50 ml screw cap tubes.
b. Note: Several good quality negative sputa can be pooled together and then split into 3 ml aliquots. Sputa should be checked for AFB prior to pooling.
c. Add 1 drop (approx. 50 µl) of 40% Formaldehyde per 1 ml of sputum, vortex well.
d. Incubate for 1 hour at room temperature (25-30°C).
e. Add 1 ml of 4% NaOH (if the sputum is too thick, add up to 2 ml of NaOH solution so that the final concentration of NaOH is always 1-2%).
f. Vortex for 2-3 min.
g. Add up to 20 ml of distilled water, mix well.
h. Incubate in a water bath for 10 min. at 55-60°C (Note: the negative specimen should be heated for a shorter period than the positive specimen to preserve white blood cells). If there is no water bath available, boil a beaker of water, cool to 90-95°C and place the tube in the beaker for 5-10 min.

This preparation is used as a diluent in the Dilution Procedure (step 7).

5. Evaluation of Positive Stock Preparations

a. If foam has formed on top of the stock solution, pipette the contents from beneath the foam into a fresh tube.

b. Using a standard microbiological loop make 2-3 test smears (approx. 1x2 cm in size) from the suspension for evaluation of the stock preparations.
c. Use a well leveled surface for drying the smears.

Positive stock: It is optimal to have concentration 50-60 AFB per microscope field.

6. Dilution Procedure

a. Using negative preparation as a diluent make dilutions according to WHO Guidelines for AFB quantification:

0 AFB/100 fields:	negative
1-9 AFB/100 fields:	exact # of AFB required
10-99 AFB/100 fields:	1+
1-10 AFB/field:	2+
>10 AFB/field :	3+

b. Choose suitable AFB concentration on a case-to-case basis within suggested range. For better results, however, it may be recommended using 20 AFB/field for 3+ smears, 5 AFB/field for 2+ smear, 50 AFB/100 fields for 1+ smears, and 5 AFB/100 fields for "exact" smears.
c. Make 3-4 ml of each suspension in order to be able to generate sufficient amount of smears.
d. For easy calculations both AFB-positive and AFB-negative aliquots are measured in drops. Calibrate one typical disposable Pasteur pipette by measuring the number of drops in 1 ml of sputum suspension. Note: do not use water for calibration since the amount of drops may be different from sputum due to the lack of viscosity.
e. For calculation of the dilution factor use the following formula :

N = (DC / AC) * A

where :

N - is amount of drops of positive sputum to be added.
DC - is desired AFB concentration.
AC - is actual AFB concentration.
A - is the amount of drops in a given volume that was estimated during calibration.

Example: AFB concentration in the stock suspension (AC) is 65 AFB/field and we have to prepare 4 ml (A = 60 drops) of 2+ suspension (DC=5 AFB/field).

In this case N = (5 AFB /65 AFB)*60 drops
N = 4.6 drops (approx 5 drops). So, 5 drops of the positive prep is mixed with 55 (60 - 5 = 55) drops of the negative prep.

Procedural notes:

1. It is important for reading and interpretation of results that appearance of the smears is more or less consistent, and that is why it would be beneficial to keep the

amount of leucocytes as stable as possible in various dilutions. In order to achieve this, it is suggested to dilute negative sputum with distilled water (prior to adding NaOH) when the amount of leukocytes is relatively high and avoid dilution if the amount of leukocytes is low.

2. It would be also useful when making 1+ suspension to consider making two different concentrations: 50 AFB/100 fields for 1+ smear preparation and 15 AFB/100 fields for further dilution to "exact" count smear.

7. Prepare and Validate Batches of Slides

a. Using diluted stock preparations, prepare slide batches (50-100 slides per batch is recommended). **Note:** If laboratories are proficient in developing consistent slides, then developing many slides from fewer samples will help to save time. Heat fixed slides should last for months if stored in a cool/dry location.

b. The consistency of each batch of slides must be validated by selecting a sample of = 6 slides from each batch to be stained and read by different technicians to document consistency. Some samples that are produced and tested will not be of sufficient consistency and should be discarded.

Apppendix C.1 Form 1: Validation Log for AFB Panel testing slide batches can be used to record results for the test slides and determine if consistency standard is acceptable.

Number of Slides made The laboratory should record how many slides were made from each sample to determine how many slides are available for test slide sets. We recommend that laboratories prepare 50-100 slides so that sufficient slides are available to put duplicate samples (one stained and one unstained) in test slide sets.

Date slides made This is the date that the test slides were produced. The length of time that slides can be stored without affecting performance has not been determined, but we estimate that 4-6 months is practical with proper storage.

Slide test results (columns 1-6) Each column represents the number AFB/100 fields for 6 separate slides selected for the sample and preferably read by 2-6 different technicians. For high positives (2+ or 3+) the technicians may estimate the number AFB/100 fields by selecting a sufficient number of representative fields. For low positives (exact count AFB/100 fields and 1+) and AFB negatives slides the technicians should read a minimum of 300 fields per slide and record the average number AFB/100 fields.

Average/Mean average is computed from slide test results 1-6 (see example)

Standard deviation The standard deviation is computed from slide test results 1-6 (see example).

$$\sqrt{\frac{n\,\Sigma x^2 - (\Sigma\, x)^2}{n(n-1)}}$$

Consistency The consistency column result is computed using the following formula:

Mean [M] minus 2 standard deviations [SD]
If M - 2 SD is > 0 then consistency is true (sufficient)
If M - 2 SD is < 0 then consistency is false (insufficient)

If the consistency is false—then there is too much variation in the number of AFB per slide and this sample is not of sufficient consistency to use in a PT test for a reliable evaluation of performance. This formula provides an objective evaluation of consistency, but the laboratory should still review and determine what is acceptable variation within a sample of slides.

Report Result This is the slide test result for all the test slides. This test result should be representative of the 6 slides tested and the sample should meet the consistency criteria.

8. Prepare Panel Testing Sets

Sets of slides with identical composition of positives and negatives can be made from the prepared batches of slides.

Appendix C.2: Logbook of Test Slide Sets can be used to select slide sets and record the original batch numbers and expected results for a 10 slide panel testing exercise. This form can also be used to record and evaluate the results from one or more peripheral laboratories that perform the PT test. Form 2 serves as the official record of results for multiple slide sets that are sent to different laboratories.

Alternate Procedure using NALC (ref Dr. Sang Jae Kim, South Korea)

a. Collection of sputum specimens: sputum specimens with numerous AFB should be collected from the patients and be stored for not more than 2 days after collection in order to prevent destruction of sputum cells. Fresh AFB negative sputa also must be selected from the routine specimens.
b. Preparation of mucolytic solution: 2% of N-acetyl-L-cysteine is mixed with an equal amount of 2.9% sodium citrate.$2H_2O$ right before use.
c. Liquefaction of sputum specimen: AFB positive and negative sputum samples are mixed with an equal amount of mucolytic solution separately and shake gently to liquefy specimens.
d. Dilution of AFB positive sputum homogenate: the liquefied AFB-positive sputum is diluted with varying proportions of AFB-negative specimen.
e. AFB counts of sputum dilutions: one drop of each sputum dilution is spread on a slide with a smear size of 2 cm^2 and dry and sterilize in a hot oven for one hour without scorching. 10 smears are prepared with each sputum dilution and stained with Ziehl-Neelsen staining method and count AFB per 1, 10 or 100 microscopic fields. Sputum dilutions whose average AFB counts fall into "1-9/100 fields", "10-99/100 fields (1+)", "1-10 per microscopic field (2+)" or "more than 10 per

microscopic field (3+)" are selected and used to prepare as many smear slides as possible. Negative slides must also be prepared. AFB counts should be rechecked with randomly selected 10 to 15 slides again after completion of smear preparation in order to confirm AFB counts of every batch of slides.

C.2: Validation Log for AFB Panel Testing Slide Batches

	Slide Preparation		Slide evaluation									
Batch No.	Number of slides made	Date Slides Made	Slide test results (AFB per 100 fields)							Standard deviation	Consistency (average minus 2 stand deviations)	Report result
			1	2	3	4	5	6	Average			
			10	10	50	15	10	11	17.7	16.0	FALSE	1+

C.3: Logbook of Test Slide Sets

(Record of a set of 10 slides selected from Form 1)

Central Laboratory administering test: _____

Slide Set No. _____

Date slide set sent to peripheral laboratories: _____/____/_____

Slide no.	Stained or unstained	Batch no.	Expected result (from Form 1.)	Peripheral Laboratory Results										Comments
				1	2	3	4	5	6	7	8	9	10	
Slide 1														
Slide 2														
Slide 3														
Slide 4														
Slide 5														
Slide 6														
Slide 7														
Slide 8														
Slide 9														
Slide 10														

C.4: Panel Testing Recording and Feedback Form

Central Laboratory Use Only:
Test Slide set #: _____
Date Sent: _____
Date results received: _____

Peripheral aboratory: _____

Date PT received by your laboratory: _____ (DD/MM/YY)

Date PT results returned to Central Laboratory: _____ (DD/MM/YY)

Name of technician reading test smears: _____

Note: If more than one technician performs AFB microscopy in the laboratory, each technician should read all 10 smears and record their results on a separate form. Technicians should not discuss results or share forms until all results have been sent back to the central laboratory. Forms for all technicians should be sent to the central laboratory for evaluation.

		Reference Laboratory Only		
Slide Number	**Result**	**Expected Result**	**Error Type**	**Points**

Feedback				
Total Points:			Pass/Fail:	
HFP	HFN	LFP	LFN	QE
Recommended Action:				

C5: Panel Testing Report of Multiple Laboratories for District Supervisor & NTP

District: _____

District Supervisor: _____

Test Slide Set: _____

Supervising Laboratory: _____

Peripheral Lab	Annual Volume	SPR	PT Score	HFN	HFP	LFN	LFP	QE	Total Errors
District Averages									

SPR :slide positivity rate PT: Panel Testing HFN: High False Negatives HFP: High False Positives
LFN: Now False Negatives LFP: Low False Positives QE: Quantitation Errors

D1: Blinded Rechecking-Parameters for Determining Sample Size

A goal of the sample size determination model proposed in this guidance is to obtain the smallest possible sample that allows conclusions about the performance of the laboratory. The widely used system of sampling 100% of positive smears and 10% of negative smears is no longer recommended for a number of reasons:

- In a well performing lab, FP are uncommon and 100% sampling of positives is unnecessary.
- In low volume laboratories the practice of rechecking 10% of negatives generally results in under-sampling.
- High volume laboratories are frequently over sampling using the 100/10 system, resulting in heavy workload and wasted resources.

In order to select a more efficient and statistically valid method, important characteristics of AFB smear microscopy were considered:

False Positives Even in high prevalence areas, the number of positive smears seen in any laboratory are relatively few, and permissible error rates are close to zero, so that often all positives would have to be rechecked to obtain statistical significance. However, any high false positive detected during rechecking is an indication of a problem and thus significant, so achieving statistical validity is not necessary. Selection of positives in the same proportion that they occur in the laboratory facilitates random and representative sampling methods. This also makes it possible to compare error rates of peripheral centers and controllers directly, for validation of the controls. FP are usually a problem in laboratories where no supervision or rechecking has been done, however, once EQA is implemented, this problem is usually resolved.

False Negatives Some false negative results are to be expected. The rate of false negatives will vary not only with the overall quality of the microscopy, but also with the positivity rate seen in the laboratory. For false negatives, rechecking should aim at discriminating between the unavoidable errors inherent in the technique, and unsatisfactory performance. This can be done by choosing a reasonable and achievable limit of false negatives, above which action is required. This threshold or upper limit for the proportion of false negatives is called the critical value.

The methods proposed here are based on the Lot Quality Assurance System (LQAS). LQAS is a method to determine an optimum sample size which when applied properly, yields statistically acceptable samples to assess quality of work, in this case, the work of the laboratory technicians. This method was originally designed for manufacturing processes where an efficient statistical model was necessary in order to keep sampling costs to a minimum. This method has been applied in health care systems to determine whether a population meets a certain standard. A number of variables are used to determine sample size using LQAS:

Lot (N) Total number of negative slides prepared in a specified period of time (one month, one quarter, one year). It is an operational quantity used to determine the sample size. Example: Lot = 5000/yr, 1250/quarter, 417/month. It is important to choose an interval of time that produces a Lot size that results in an economical and statistically valid sample. If the Lot size is too small, this may not be possible. It is also important to note that although N is the number used for determining sample size for a specified time interval for the purpose of making a valid conclusion for that interval, the actual collection of the sample and rechecking by the controllers can be done more frequently to reduce the possibility of slides being lost, or fading. In this example, the Lot size 5000/year may result in the most efficient sample size, but the total sample size could be achieved by cumulatively collecting one quarter of the total sample during each of the quarterly supervisor visits.

Critical Value An upper threshold of the proportion of false negatives among all the negatives beyond which intervention is deemed necessary. Critical value can be chosen from an estimate of the historical (long term) false negativity rates, but in the early stages of an EQA program, accurate data may not be available. The critical value can be calculated based on the prevalence of positives, and expected parameters for sensitivity and specificity (relative to the controllers) as defined by the program. A table of calculated Critical Values as a function of sensitivity, specificity and positivity rate is available as Appendix D.1, along with an example of how critical values are determined. For the purposes of this manual, the critical value has been determined based on prevalence of positives and the expected sensitivity.

Acceptance Number (d) The maximum number of false negative errors allowed in the sample after which the NTP/NRL can no longer be certain that the expected performance has been achieved. The value chosen for "d" has a direct impact on sample size, the larger the acceptance number, the larger the sample size required. In order to achieve the smallest, most efficient sample size, a value of d=0 is recommended. As previously described, for the purpose of efficiency all error types, including LFN and HFN, are included for the determination of sample size. Although this implies that even one error exceeds the threshold for action, the fact that some proportion of false negatives is expected has been built into the calculation (critical value), so that the zero threshold represents false negative rates above the expected proportion in the Lot of smears. Therefore, the finding of a single error detected can be considered a warning of a possible problem and should be investigated. However, finding an error does not prove that there is a real problem and investigation may indicate that this was a chance detection of a random error in fact below the critical value or false alarm. Larger numbers of errors detected will be more likely to represent a true problem in performance. Since both major and minor errors are included in the calculation of sample size, the interpretation of errors and the appropriate action should depend on both the number and the type or errors, and their evolution in time, as well as the resources of the NTP to implement corrective action.

Slide Positivity rate (SPR) The SPR is the proportion of positive smears among all slides (diagnostic and follow-up) in the laboratory from which the sample is to be taken. This number is estimated using the laboratory registers from the previous year. Sample sizes should be determined using the average positivity rate for an area or country since precision at the level of each laboratory may not be necessary or practical.

$$\text{SPR} = \frac{\text{Number of positive smears per year}}{\text{Annual slide volume}} \times 100$$

Sensitivity Ability of the technician to detect AFB relative to the controllers[**]. It is important to remember that even a controller will never achieve 100% sensitivity. Relative sensitivity for high positives (2-3+) should be close to 95%, but may be as low as 30-50% for low positives (1-9 AFB/100 fields). For this reason, the program will need to select a sensitivity based on reasonable expected overall performance. Since both major and minor errors are to be considered in the determination of sample size using this model, an overall sensitivity of 75-85% is recommended. If only HFN were included in the sample calculation, a sensitivity of at least 95% would be expected, resulting in a lower Critical Value, and ultimately in a substantially greater sample size. This would most likely limit the feasibility of implementing a blinded rechecking program in many settings. New programs may want to start by using a sensitivity of 75-80% as this will reduce the sample size significantly, which may help to make implementation of a rechecking program more feasible. This will also allow programs to focus corrective action on laboratories where performance is very poor. As the program obtains additional resources, and as overall performance is expected to improve, the sensitivity used to determine sample size should be increased to 80 or even 85%.

Specificity Set at 100% because any false positive should trigger action. One limitation of this method is that the sample of positives is too small to allow any conclusion about whether the desired specificity has been met if no false positives are found.

Confidence Interval All of the sample sizes have been developed to determine if the laboratory has met the expected sensitivity within a 95% confidence level. Therefore, if the d=0 and there are no false negatives detected within the sample then the NRL can determine with a 95% confidence level that the peripheral laboratory is performing at or above the acceptable sensitivity.

Calculation of sample size

In simple terms the calculation of sample size is based on the population of negative slides and the calculated sample size is adjusted, or increased proportional to the positivity rate to yield a sample size of positive and negative smears. Slides are collected from the entire lot of slides irrespective of whether the result was positive or negative.

[**] This should not be confused with sensitivity of smear compared to culture, which is used as the gold standard.

The method of random sampling will assure that the number of positive, negative, false negative, and false positive slides in the sample is representative of the entire set of slides processed by the laboratory. In centers with very low slide positivity rates the sample may occasionally contain few if any positives, so that rechecking would not be useful to detect False Positives. In laboratories where this is a concern, it may be necessary to modify the collection scheme to include an additional number of positive and scanty slides for rechecking.

One important distinction of this approach is that the sample size of negatives is based on LQAS and the presence or lack of errors provides an indication about whether the laboratory has met a pre-determined goal for test sensitivity.

Using a d=0 and a predetermined performance goal (such as 80% sensitivity), if a laboratory has no false negatives then there is assurance within a 95% confidence interval that the laboratory has met the sensitivity goal.

The number of positives within the sample size is not based on LQAS, but rather the number is chosen based on the proportion they occur in the laboratory. Using LQAS for positives would involve a much larger sample size and require separate sampling of positives and negatives. Separate sampling of positives is not practical when using random sampling and the large sample size may be unnecessary to detect systematic problems of misinterpreting debris, precipitates or other material as AFB. Therefore, within the sample collected from a laboratory the negatives represent a statistical sample size that is measured against d=0 and the positives are a merely a sample. Any error within the sample may represent a problem and will need further evaluation. The presence of some false negative(s) indicates a laboratory may not be meeting a performance goal of sensitivity and any false positive within a small sample may indicate a systematic problem. This approach allows the supervising laboratory to collect a small combined sample of positives and negatives and make some conclusions about performance. This combined sample provides a balance between rigorous statistical sampling and the need to provide a small sample that simplifies implementation and increase the chances of sustaining a rechecking program.

The tables in Appendix D.3 can be used to determine sample size based on a range of Lot sizes and positivity rates. Simple tables are included for acceptance number d=0 and d=1 so that laboratories can evaluate the implications of the increase in sample size when d=1 us used. Simple tables are presented for sensitivities of 65% 70%, 75%, 80%, 85% or 90%. All the sample sizes shown reflect total sample to be collected

For programs that want to take a more detailed approach to determining sample size based on a narrower range of Lot sizes, positivity rates, or consider increasing the acceptance number, more detailed tables are provided in Appendix D.4 for sensitivities of 65% 70%, 75%, 80%, 85% or 90%. All of the sample sizes shown reflect total sample to be collected.

D.2: Critical values

CV as function of smear sensitivity, specificity and prevalence of positives

Specificity kept at 100%

	Sensitivity					
Positivity rate	**65%**	**70%**	**75%**	**80%**	**85%**	**90%**
0.50%	0.27%	0.22%	0.17%	0.13%	0.09%	0.06%
1.00%	0.54%	0.43%	0.34%	0.25%	0.18%	0.11%
2.00%	1.10%	0.87%	0.68%	0.51%	0.36%	0.23%
2.50%	1.38%	1.10%	0.85%	0.64%	0.45%	0.28%
3.00%	1.67%	1.33%	1.03%	0.77%	0.55%	0.34%
4.00%	2.24%	1.79%	1.39%	1.04%	0.74%	0.46%
5.00%	2.83%	2.26%	1.75%	1.32%	0.93%	0.58%
6.00%	3.44%	2.74%	2.13%	1.60%	1.13%	0.71%
7.00%	4.05%	3.23%	2.51%	1.88%	1.33%	0.84%
7.50%	4.37%	3.47%	2.70%	2.03%	1.43%	0.90%
8.00%	4.68%	3.73%	2.90%	2.17%	1.53%	0.97%
9.00%	5.33%	4.24%	3.30%	2.47%	1.75%	1.10%
10.00%	5.98%	4.76%	3.70%	2.78%	1.96%	1.23%
11.00%	6.66%	5.30%	4.12%	3.09%	2.18%	1.37%
12.00%	7.34%	5.84%	4.55%	3.41%	2.41%	1.52%
13.00%	8.05%	6.40%	4.98%	3.74%	2.64%	1.66%
14.00%	8.77%	6.98%	5.43%	4.07%	2.87%	1.81%
15.00%	9.50%	7.56%	5.88%	4.41%	3.11%	1.96%
16.00%	10.26%	8.16%	6.35%	4.76%	3.36%	2.12%
17.00%	11.03%	8.78%	6.83%	5.12%	3.61%	2.28%
18.00%	11.82%	9.41%	7.32%	5.49%	3.87%	2.44%
19.00%	12.63%	10.05%	7.82%	5.86%	4.14%	2.61%
20.00%	13.46%	10.71%	8.33%	6.25%	4.41%	2.78%
21.00%	14.31%	11.39%	8.86%	6.65%	4.69%	2.95%
22.00%	15.19%	12.09%	9.40%	7.05%	4.98%	3.13%
23.00%	16.08%	12.80%	9.96%	7.47%	5.27%	3.32%
24.00%	17.00%	13.53%	10.53%	7.89%	5.57%	3.51%
25.00%	17.95%	14.29%	11.11%	8.33%	5.88%	3.70%
26.00%	18.92%	15.06%	11.71%	8.78%	6.20%	3.90%
27.00%	19.92%	15.85%	12.33%	9.25%	6.53%	4.11%
28.00%	20.94%	16.67%	12.96%	9.72%	6.86%	4.32%
29.00%	21.99%	17.51%	13.62%	10.21%	7.21%	4.54%
30.00%	23.08%	18.37%	14.29%	10.71%	7.56%	4.76%
31.00%	24.19%	19.25%	14.98%	11.23%	7.93%	4.99%
32.00%	25.34%	20.17%	15.69%	11.76%	8.30%	5.23%
33.00%	26.52%	21.11%	16.42%	12.31%	8.69%	5.47%
34.00%	27.74%	22.08%	17.17%	12.88%	9.09%	5.72%
35.00%	28.99%	23.08%	17.95%	13.46%	9.50%	5.98%

Calculation of Critical Value

Examples of the calculation of critical value for sensitivity of 60-90%, and specificity of 100% for a positivity rate of 15% are shown in this table:

Start From:

Expected sensitivity and specificity relative to the controllers
Positivity rate in the labs controlled determine FP and FN allowed using a cross-table FN and FP constitute the critical values

Positivity rate 15%

Sensitivity: 50.00% Specificity: 100.00%

		Controllers		
		+	**-**	**Total**
Results being rechecked	**+**	150	0	150
	-	150	700	850
	Total	300	700	1000

Critical values: **FP** **0.00%** **FN** **17.65%**

Sensitivity: 55.00% Specificity: 100.00%

		Controllers		
		+	**-**	Total
Results being rechecked	**+**	150	0	150
	-	122.73	727.27	850
	Total	272.73	727.27	1000

Critical values: **FP** **0.00%** **FN** **14.44%**

Sensitivity: 60.00% Specificity: 100.00%

		Controllers +	Controllers -	**Total**
Results being rechecked	+	150	0	150
	-	100	750	850
	Total	250	750	1000

Critical values: **FP** **0.00%** **FN** **11.76%**

Sensitivity: 65.00% Specificity: 100.00%

		Controllers +	Controllers -	Total
Results being rechecked	+	150	0	150
	-	80.77	769.23	850
	Total	230.77	769.23	1000

Critical values: **FP** **0.00%** **FN** **9.50%**

Sensitivity: 70.00% Specificity: 100.00%

		Controllers +	Controllers -	Total
Results being rechecked	+	150	0	150
	-	64.29	785.71	850
	Total	214.29	785.71	1000

Critical values: **FP** **0.00%** **FN** **7.56%**

Sensitivity: 75.00% Specificity: 100.00%

		Controllers		
		+	-	Total
Results being rechecked	+	150	0	150
	-	50	800	850
	Total	200	800	1000

Critical values: **FP** **0.00%** **FN** **5.88%**

Sensitivity: 80.00% Specificity: 100.00%

		Controllers		
		+	-	Total
Results being rechecked	+	150	0	150
	-	37.5	812.5	850
	Total	187.5	812.5	1000

Critical values: **FP** **0.00%** **FN** **4.41%**

Sensitivity: 85.00% Specificity: 100.00%

		Controllers		
		+	-	Total
Results being rechecked	+	150	0	150
	-	26.47	823.53	850
	Total	176.47	823.53	1000

Critical values: **FP** **0.00%** **FN** **3.11%**

Sensitivity: 90.00% Specificity: 100.00%

		Controllers		
		+	-	Total
Results being rechecked	+	150	0	150
	-	16.67	833.33	850
	Total	166.67	833.33	1000

Critical values: **FP** **0.00%** **FN** **1.96%**

D3: Simple Sample Size Tables

LQAS sample size table (Simplified)

Sensitivity (relative to controllers) = **65%**
Specificity = 100% Acceptance number d = 0

Number of	Slide positivity rate					
negative slides/year	5%	10%	15%	20%	25%	30%
200	68	40	27	21	16	13
500	84	44	29	21	17	13
1000	91	46	31	23	17	13
5000	98	48	31	23	17	13
50000	99	48	31	23	17	14

Sensitivity (relative to controllers) = **65%**
Specificity = 100% Acceptance number d = 1

Number of	Slide positivity rate					
negative slides/year	5%	10%	15%	20%	25%	30%
200	111	66	47	35	28	23
500	139	73	51	38	29	24
1000	152	77	52	38	29	24
5000	163	80	53	39	29	24
50000	165	80	53	39	31	24

Sensitivity (relative to controllers) = **70%**
Specificity = 100% Acceptance number d = 0

Number of	Slide positivity rate					
negative slides/year	5%	10%	15%	20%	25%	30%
200	78	48	34	26	21	17
500	99	54	36	28	21	17
1000	109	57	38	29	21	17
5000	119	60	39	29	23	17
50000	121	60	39	29	23	17

Sensitivity (relative to controllers) = **70%**
Specificity = 100% Acceptance number d = 1

Number of	Slide positivity rate					
negative slides/year	5%	10%	15%	20%	25%	30%
200	125	79	56	44	36	30
500	163	91	62	48	37	30
1000	181	96	65	49	39	31
5000	198	100	66	49	39	31
50000	202	101	67	50	39	31

D.3: Simple Sample Size Tables

LQAS sample size table (Simplified)

Sensitivity (relative to controllers) = 75%
Specificity = 100% Acceptance number d = 0

Number of negative slides/year	Slide positivity rate 5%	10%	15%	20%	25%	30%
200	91	59	42	34	27	23
500	121	69	47	36	28	23
1000	136	73	49	38	29	23
5000	152	78	51	38	29	24
50000	156	79	52	38	29	24

Sensitivity (relative to controllers) = 75%
Specificity = 100% Acceptance number d = 1

Number of negative slides/year	Slide positivity rate 5%	10%	15%	20%	25%	30%
200	143	96	71	56	45	39
500	198	114	80	61	48	40
1000	224	123	82	63	49	41
5000	252	130	86	64	51	41
50000	259	132	87	65	51	41

Sensitivity (relative to controllers) = 80%
Specificity = 100% Acceptance number d = 0

Number of negative slides/year	Slide positivity rate 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
50000	216	104	69	51	40	33

Sensitivity (relative to controllers) = 80%
Specificity = 100% Acceptance number d = 1

Number of negative slides/year	Slide positivity rate 5%	10%	15%	20%	25%	30%
200	167	117	89	71	60	50
500	251	147	105	79	65	54
1000	296	160	111	83	67	56
5000	345	172	115	85	68	56
50000	359	174	116	86	69	57

D.3: Simple Sample Size Tables

LQAS sample size table (Simplified)

Sensitivity (relative to controllers) = **85%**
Specificity = 100% Acceptance number d = 0

Number of negative slides/year	Slide positivity rate 5%	10%	15%	20%	25%	30%
200	126	89	71	58	48	41
500	197	117	86	66	53	44
1000	242	131	93	70	56	46
5000	297	144	99	74	57	47
50000	313	148	100	74	59	47

Sensitivity (relative to controllers) = **85%**
Specificity = 100% Acceptance number d = 1

Number of negative slides/year	Slide positivity rate 5%	10%	15%	20%	25%	30%
200	192	143	115	95	80	69
500	317	192	142	111	91	76
1000	396	217	154	118	93	79
5000	491	240	165	123	97	80
50000	519	246	167	124	99	81

Sensitivity (relative to controllers) = **90%**
Specificity = 100% Acceptance number d = 0

Number of negative slides/year	Slide positivity rate 5%	10%	15%	20%	25%	30%
200	146	118	94	81	71	61
500	249	172	124	100	83	70
1000	326	203	139	108	88	73
5000	434	238	153	116	93	77
50000	468	247	156	118	95	77

Sensitivity (relative to controllers) = **90%**
Specificity = 100% Acceptance number d = 1

Number of negative slides/year	Slide positivity rate 5%	10%	15%	20%	25%	30%
200	208	183	152	131	115	101
500	393	279	204	165	137	117
1000	528	333	229	180	148	123
5000	716	394	254	194	156	129
50000	777	410	260	196	159	130

D.4: Sensitivity Relative to the Controllers At 65%

		Positivity Rate						
		2.5%	5.0%	7.5%	10.0%	13.0%	15.0%	18.0%
Negatives Examined Annually	Acceptance Number	Total Sample Required						
100	d=0	68	52	41	33	28	25	21
200	d=0	101	68	50	40	31	27	23
300	d=0	120	76	54	42	32	28	24
400	d=0	133	81	56	43	33	29	24
500	d=0	143	84	57	44	33	29	24
700	d=0	154	87	59	46	34	29	24
1000	d=0	165	91	61	46	34	31	24
2000	d=0	179	95	63	47	36	31	26
5000	d=0	189	98	64	48	36	31	26
10000	d=0	193	99	64	48	36	31	26
20000	d=0	194	99	64	48	36	31	26
50000	d=0	195	99	64	48	36	31	26
100	d=1	99	81	66	56	46	41	35
200	d=1	158	111	82	66	53	47	39
300	d=1	193	124	90	70	55	48	40
400	d=1	215	133	93	72	56	49	41
500	d=1	232	139	96	73	57	51	41
700	d=1	252	145	98	76	59	51	43
1000	d=1	271	152	102	77	59	52	43
2000	d=1	296	158	105	79	60	52	43
5000	d=1	314	163	106	80	61	53	43
10000	d=1	320	164	107	80	61	53	44
20000	d=1	323	165	107	80	61	53	44
50000	d=1	325	165	107	80	61	53	44
100	d=2	103	100	84	72	61	55	48
200	d=2	194	143	108	88	70	62	54
300	d=2	246	164	119	94	75	66	55
400	d=2	279	177	124	98	76	67	56
500	d=2	303	184	129	100	77	68	57
700	d=2	334	195	133	102	79	69	57
1000	d=2	361	203	136	104	80	69	59
2000	d=2	397	214	142	107	82	71	59
5000	d=2	422	220	144	108	83	72	59
10000	d=2	431	222	145	109	83	72	60
20000	d=2	436	223	145	109	83	72	60
50000	d=2	438	224	146	109	83	72	60
100	d=3	103	105	98	87	74	67	60
200	d=3	205	171	132	108	87	78	66
300	d=3	285	199	146	116	92	81	68
400	d=3	332	216	154	120	94	84	70
500	d=3	364	226	159	123	97	85	71
700	d=3	406	240	164	127	98	86	72
1000	d=3	442	251	170	129	100	87	72
2000	d=3	490	264	175	132	101	88	73
5000	d=3	523	274	179	134	102	89	74
10000	d=3	535	276	181	136	103	89	74
20000	d=3	541	278	181	136	103	89	74
50000	d=3	545	279	182	136	103	89	74
100	d=4	103	105	107	98	85	79	70
200	d=4	205	193	152	126	102	92	78
300	d=4	308	229	171	137	108	96	82
400	d=4	374	252	181	142	111	99	83
500	d=4	417	265	187	146	114	100	84
700	d=4	472	282	195	150	116	102	85
1000	d=4	518	296	201	153	118	104	87
2000	d=4	578	314	208	158	121	105	88
5000	d=4	619	324	213	160	122	106	88
10000	d=4	635	328	214	161	123	107	88
20000	d=4	642	329	215	161	123	107	88
50000	d=4	647	332	215	161	123	107	89

D.4: Sensitivity Relative to the Controllers At 65%

		Positivity Rate						
		20.0%	23.0%	25.0%	28.0%	30.0%	33.0%	35.0%
Negatives Examined Annually	Acceptance Number	Total Sample Required						
100	d=0	19	17	16	14	13	12	11
200	d=0	21	18	16	14	13	12	11
300	d=0	21	18	16	14	13	12	11
400	d=0	21	18	16	15	13	12	11
500	d=0	21	18	17	15	13	12	11
700	d=0	21	18	17	15	13	12	11
1000	d=0	23	18	17	15	13	12	11
2000	d=0	23	19	17	15	13	12	11
5000	d=0	23	19	17	15	13	12	11
10000	d=0	23	19	17	15	13	12	11
20000	d=0	23	19	17	15	14	12	11
50000	d=0	23	19	17	15	14	12	11
100	d=1	33	29	27	24	23	21	18
200	d=1	35	31	28	25	23	21	20
300	d=1	36	31	29	25	24	21	20
400	d=1	38	32	29	26	24	21	20
500	d=1	38	32	29	26	24	21	20
700	d=1	38	32	29	26	24	21	20
1000	d=1	38	32	29	26	24	21	20
2000	d=1	39	32	29	26	24	21	20
5000	d=1	39	32	29	26	24	22	20
10000	d=1	39	32	31	26	24	22	20
20000	d=1	39	32	31	26	24	22	20
50000	d=1	39	32	31	26	24	22	20
100	d=2	44	39	36	33	30	28	26
200	d=2	48	42	39	35	31	28	28
300	d=2	50	43	40	35	33	30	28
400	d=2	50	44	40	36	33	30	28
500	d=2	51	44	40	36	33	30	28
700	d=2	51	44	40	36	33	30	28
1000	d=2	51	44	41	36	33	30	28
2000	d=2	53	45	41	36	33	30	28
5000	d=2	53	45	41	36	34	30	28
10000	d=2	53	45	41	36	34	30	28
20000	d=2	53	45	41	36	34	30	28
50000	d=2	53	45	41	36	34	30	28
100	d=3	54	48	45	40	39	34	32
200	d=3	60	52	48	43	40	36	34
300	d=3	61	55	49	44	41	37	34
400	d=3	63	55	51	44	41	37	35
500	d=3	64	55	51	44	41	37	35
700	d=3	64	56	51	46	41	37	35
1000	d=3	65	56	51	46	41	37	35
2000	d=3	65	56	52	46	43	37	35
5000	d=3	66	57	52	46	43	37	35
10000	d=3	66	57	52	46	43	39	35
20000	d=3	66	57	52	46	43	39	35
50000	d=3	66	57	52	46	43	39	35
100	d=4	64	57	53	49	46	42	38
200	d=4	71	62	57	51	49	43	42
300	d=4	74	64	59	53	49	45	42
400	d=4	75	65	60	53	50	45	42
500	d=4	75	66	60	54	50	45	42
700	d=4	76	66	61	54	50	45	42
1000	d=4	78	66	61	54	50	45	42
2000	d=4	78	68	61	54	50	45	43
5000	d=4	79	68	61	56	51	46	43
10000	d=4	79	68	61	56	51	46	43
20000	d=4	79	68	63	56	51	46	43
50000	d=4	79	68	63	56	51	46	43

D.4: Sensitivity Relative to the Controllers At 70%

		Positivity Rate						
		2.5%	5.0%	7.5%	10.0%	13.0%	15.0%	18.0%
Negatives Examined Annually	Acceptance Number	Total Sample Required						
100	d=0	74	57	46	40	33	29	26
200	d=0	114	78	59	48	39	34	29
300	d=0	138	88	65	51	41	35	30
400	d=0	156	95	69	53	43	36	30
500	d=0	168	99	71	54	43	36	30
700	d=0	186	104	74	56	44	38	32
1000	d=0	201	109	76	57	45	38	32
2000	d=0	223	115	78	59	45	39	32
5000	d=0	239	119	80	60	46	39	32
10000	d=0	244	120	81	60	46	39	33
20000	d=0	247	121	81	60	46	39	33
50000	d=0	249	121	81	60	46	39	33
100	d=1	103	88	75	64	55	49	44
200	d=1	174	125	97	79	64	56	49
300	d=1	219	144	108	86	69	60	51
400	d=1	250	155	114	89	70	61	51
500	d=1	273	163	118	91	72	62	52
700	d=1	303	173	122	93	74	64	54
1000	d=1	330	181	126	96	75	65	54
2000	d=1	368	192	131	99	76	66	55
5000	d=1	395	198	134	100	77	66	55
10000	d=1	405	200	135	101	77	66	55
20000	d=1	410	201	135	101	78	67	55
50000	d=1	413	202	136	101	78	67	55
100	d=2	103	104	94	82	71	65	57
200	d=2	204	160	128	104	86	76	66
300	d=2	274	188	143	113	92	80	68
400	d=2	320	205	151	119	95	82	71
500	d=2	353	216	157	122	97	85	71
700	d=2	398	231	164	127	99	86	72
1000	d=2	437	242	170	129	101	87	73
2000	d=2	492	257	176	133	103	89	74
5000	d=2	531	267	181	136	105	89	74
10000	d=2	546	271	183	137	105	91	76
20000	d=2	554	273	183	137	106	91	76
50000	d=2	558	274	184	137	106	91	76
100	d=3	103	105	106	98	86	79	71
200	d=3	205	187	154	127	106	94	80
300	d=3	306	226	174	140	114	100	85
400	d=3	373	249	186	147	117	102	87
500	d=3	418	264	194	151	121	105	89
700	d=3	480	283	202	157	123	107	90
1000	d=3	533	299	210	160	125	108	91
2000	d=3	607	319	219	166	129	111	93
5000	d=3	658	332	225	169	130	112	94
10000	d=3	677	336	227	170	131	113	94
20000	d=3	687	338	228	170	131	113	94
50000	d=3	693	340	228	170	131	113	94
100	d=4	103	105	108	108	99	91	82
200	d=4	205	206	176	148	123	111	95
300	d=4	308	259	202	163	133	118	101
400	d=4	406	288	217	172	139	121	104
500	d=4	472	308	227	178	143	124	105
700	d=4	552	333	239	184	146	127	107
1000	d=4	621	352	249	190	149	129	109
2000	d=4	714	377	259	197	153	132	110
5000	d=4	779	394	267	200	155	133	111
10000	d=4	803	399	269	201	156	134	112
20000	d=4	815	402	270	202	156	134	112
50000	d=4	823	403	271	202	156	134	112

D.4: Sensitivity Relative to the Controllers At 70%

Negatives Examined Annually	Acceptance Number	Positivity Rate 20.0%	23.0%	25.0%	28.0%	30.0%	33.0%	35.0%
		Total Sample Required						
100	d=0	24	21	20	17	16	15	14
200	d=0	26	22	21	18	17	15	14
300	d=0	28	23	21	18	17	15	14
400	d=0	28	23	21	19	17	15	14
500	d=0	28	23	21	19	17	15	14
700	d=0	28	23	21	19	17	15	14
1000	d=0	29	25	21	19	17	16	14
2000	d=0	29	25	23	19	17	16	14
5000	d=0	29	25	23	19	17	16	14
10000	d=0	29	25	23	19	17	16	14
20000	d=0	29	25	23	19	17	16	15
50000	d=0	29	25	23	19	17	16	15
100	d=1	40	35	33	31	29	25	25
200	d=1	44	39	36	32	30	27	25
300	d=1	46	40	36	32	30	27	26
400	d=1	46	40	37	33	30	27	26
500	d=1	48	40	37	33	30	28	26
700	d=1	48	42	37	33	31	28	26
1000	d=1	49	42	39	33	31	28	26
2000	d=1	49	42	39	33	31	28	26
5000	d=1	49	43	39	33	31	28	26
10000	d=1	49	43	39	33	31	28	26
20000	d=1	49	43	39	33	31	28	26
50000	d=1	50	43	39	33	31	28	26
100	d=2	54	48	45	40	39	34	32
200	d=2	60	52	48	43	40	37	34
300	d=2	63	55	49	44	41	37	35
400	d=2	64	55	51	44	41	37	35
500	d=2	64	56	51	46	41	37	35
700	d=2	65	56	52	46	43	39	35
1000	d=2	66	57	52	46	43	39	35
2000	d=2	66	57	52	46	43	39	35
5000	d=2	68	57	53	47	43	39	37
10000	d=2	68	58	53	47	43	39	37
20000	d=2	68	58	53	47	43	39	37
50000	d=2	68	58	53	47	43	39	37
100	d=3	65	58	55	50	47	43	42
200	d=3	74	65	60	54	50	46	43
300	d=3	78	68	63	56	51	48	45
400	d=3	79	69	63	57	53	48	45
500	d=3	80	70	64	57	53	48	45
700	d=3	81	70	64	57	53	48	45
1000	d=3	83	71	65	58	53	48	45
2000	d=3	84	71	65	58	54	49	46
5000	d=3	84	73	67	58	54	49	46
10000	d=3	84	73	67	58	54	49	46
20000	d=3	84	73	67	58	54	49	46
50000	d=3	84	73	67	58	54	49	46
100	d=4	76	69	65	60	56	52	49
200	d=4	88	78	72	64	60	55	52
300	d=4	91	81	75	67	61	57	52
400	d=4	94	82	75	67	63	57	54
500	d=4	95	83	76	68	63	57	54
700	d=4	96	84	77	68	63	58	54
1000	d=4	98	84	77	69	64	58	54
2000	d=4	99	86	79	69	64	58	54
5000	d=4	100	86	79	69	64	58	55
10000	d=4	100	87	79	69	64	58	55
20000	d=4	100	87	79	69	64	58	55
50000	d=4	100	87	79	69	64	58	55

D.4: Sensitivity Relative to the Controllers At 75%

		Positivity Rate						
		2.5%	5.0%	7.5%	10.0%	13.0%	15.0%	18.0%
Negatives Examined Annually	Acceptance Number	Total Sample Required						
100	d=0	78	63	54	47	40	36	32
200	d=0	123	91	71	59	48	42	37
300	d=0	154	105	80	64	52	45	38
400	d=0	175	115	85	68	53	47	39
500	d=0	192	121	89	69	54	47	40
700	d=0	215	129	93	72	55	48	40
1000	d=0	236	136	96	73	56	49	41
2000	d=0	267	145	102	77	59	51	41
5000	d=0	289	152	104	78	59	51	43
10000	d=0	297	154	105	78	60	51	43
20000	d=0	302	155	106	79	60	52	43
50000	d=0	305	156	106	79	60	52	43
100	d=1	103	96	85	74	64	59	52
200	d=1	187	143	116	96	79	71	61
300	d=1	241	169	132	106	85	75	65
400	d=1	280	186	141	111	89	78	66
500	d=1	309	198	147	114	91	80	67
700	d=1	349	213	155	119	93	81	68
1000	d=1	386	224	160	123	95	82	70
2000	d=1	439	241	169	128	98	85	71
5000	d=1	478	252	173	130	99	86	72
10000	d=1	493	256	175	131	100	86	72
20000	d=1	501	258	176	131	100	86	72
50000	d=1	506	259	176	132	100	87	72
100	d=2	103	105	104	94	84	78	70
200	d=2	205	181	150	127	105	94	82
300	d=2	293	220	173	140	114	101	87
400	d=2	353	244	186	149	118	105	89
500	d=2	395	261	196	154	122	107	90
700	d=2	454	282	206	160	125	109	93
1000	d=2	509	300	215	166	129	112	94
2000	d=2	586	324	227	172	132	115	96
5000	d=2	643	340	235	176	134	116	98
10000	d=2	664	345	237	178	136	116	98
20000	d=2	675	347	238	178	136	118	98
50000	d=2	682	349	239	179	136	118	98
100	d=3	103	105	108	108	99	93	84
200	d=3	205	205	178	152	128	115	100
300	d=3	308	261	210	171	140	125	107
400	d=3	400	294	227	182	146	129	111
500	d=3	462	316	239	190	151	133	112
700	d=3	544	345	254	198	155	136	116
1000	d=3	616	369	266	204	160	139	117
2000	d=3	720	400	281	213	164	142	120
5000	d=3	796	421	291	219	168	145	121
10000	d=3	824	428	294	220	169	146	122
20000	d=3	838	433	296	221	169	146	122
50000	d=3	846	435	297	222	169	146	122
100	d=4	103	105	108	111	110	105	96
200	d=4	205	211	201	174	148	134	118
300	d=4	308	293	241	200	164	146	127
400	d=4	410	337	265	214	172	153	130
500	d=4	506	366	280	223	178	156	133
700	d=4	618	403	298	233	184	161	137
1000	d=4	714	434	314	242	189	165	139
2000	d=4	846	473	333	253	195	169	143
5000	d=4	941	499	345	260	199	173	144
10000	d=4	975	508	349	262	200	173	145
20000	d=4	994	514	351	263	201	174	145
50000	d=4	1005	516	352	263	201	174	145

D.4: Sensitivity Relative to the Controllers At 75%

Negatives Examined Annually	Acceptance Number	Positivity Rate 20.0%	23.0%	25.0%	28.0%	30.0%	33.0%	35.0%
		Total Sample Required						
100	d=0	30	26	24	22	21	19	18
200	d=0	34	29	27	24	23	19	18
300	d=0	35	30	28	25	23	21	18
400	d=0	35	31	28	25	23	21	18
500	d=0	36	31	28	25	23	21	20
700	d=0	36	31	29	25	23	21	20
1000	d=0	38	31	29	25	23	21	20
2000	d=0	38	32	29	25	24	21	20
5000	d=0	38	32	29	26	24	21	20
10000	d=0	38	32	29	26	24	21	20
20000	d=0	38	32	29	26	24	21	20
50000	d=0	38	32	29	26	24	21	20
100	d=1	49	44	41	38	36	33	31
200	d=1	56	49	45	40	39	34	32
300	d=1	59	51	47	42	39	36	34
400	d=1	60	52	48	43	40	36	34
500	d=1	61	52	48	43	40	36	34
700	d=1	61	53	49	43	40	36	34
1000	d=1	63	53	49	44	41	37	34
2000	d=1	64	55	51	44	41	37	34
5000	d=1	64	55	51	44	41	37	34
10000	d=1	64	55	51	44	41	37	35
20000	d=1	65	55	51	44	41	37	35
50000	d=1	65	55	51	44	41	37	35
100	d=2	65	58	56	50	49	45	42
200	d=2	75	66	61	56	51	48	45
300	d=2	79	69	64	57	53	49	46
400	d=2	81	70	65	58	54	49	46
500	d=2	83	71	65	58	54	49	46
700	d=2	84	73	67	60	56	51	46
1000	d=2	85	73	68	60	56	51	48
2000	d=2	86	74	68	60	56	51	48
5000	d=2	88	75	69	61	57	51	48
10000	d=2	88	75	69	61	57	51	48
20000	d=2	88	75	69	61	57	51	48
50000	d=2	88	75	69	61	57	51	48
100	d=3	79	71	68	63	59	55	52
200	d=3	93	82	76	69	64	60	55
300	d=3	98	86	80	71	67	61	57
400	d=3	100	87	81	72	67	61	58
500	d=3	103	88	83	74	69	63	58
700	d=3	104	90	84	74	69	63	58
1000	d=3	106	91	84	75	70	63	58
2000	d=3	108	92	85	75	70	64	60
5000	d=3	109	94	87	76	71	64	60
10000	d=3	109	94	87	76	71	64	60
20000	d=3	110	94	87	76	71	64	60
50000	d=3	110	94	87	76	71	64	60
100	d=4	91	83	80	74	70	64	62
200	d=4	109	96	91	82	77	70	66
300	d=4	115	101	95	85	80	73	68
400	d=4	119	104	96	86	80	73	69
500	d=4	121	105	97	88	81	75	69
700	d=4	124	108	99	89	83	75	71
1000	d=4	126	109	100	89	83	75	71
2000	d=4	128	110	101	90	84	76	71
5000	d=4	130	112	103	90	84	76	71
10000	d=4	130	112	103	90	84	76	71
20000	d=4	130	112	103	92	84	76	72
50000	d=4	130	112	103	92	84	76	72

D.4: Sensitivity Relative to the Controllers At 80%

		Positivity Rate						
		2.5%	5.0%	7.5%	10.0%	13.0%	15.0%	18.0%
Negatives Examined Annually	Acceptance Number	Total Sample Required						
100	d=0	84	72	63	54	48	45	39
200	d=0	143	107	86	72	61	54	46
300	d=0	185	129	101	80	67	59	50
400	d=0	217	143	108	86	70	61	51
500	d=0	243	154	114	89	71	62	52
700	d=0	281	167	121	92	75	65	54
1000	d=0	318	180	128	96	76	66	55
2000	d=0	376	197	135	100	79	68	56
5000	d=0	423	208	141	103	80	69	57
10000	d=0	441	213	142	104	80	69	57
20000	d=0	450	215	143	104	82	69	57
50000	d=0	456	216	144	104	82	69	57
100	d=1	103	103	95	86	77	72	65
200	d=1	203	167	139	117	99	89	78
300	d=1	280	206	162	131	109	98	83
400	d=1	337	232	177	140	115	101	87
500	d=1	383	251	187	147	118	105	88
700	d=1	449	275	200	153	123	107	90
1000	d=1	515	296	211	160	128	111	91
2000	d=1	616	325	224	167	132	114	94
5000	d=1	697	345	234	172	134	115	95
10000	d=1	729	353	237	173	136	116	96
20000	d=1	747	357	238	174	136	116	96
50000	d=1	757	359	239	174	137	116	96
100	d=2	103	105	109	106	98	92	84
200	d=2	205	203	177	151	131	118	104
300	d=2	308	261	212	173	145	129	111
400	d=2	403	300	232	187	154	135	116
500	d=2	473	326	248	194	160	140	118
700	d=2	573	362	266	206	166	145	122
1000	d=2	670	394	281	214	171	148	124
2000	d=2	817	436	302	226	178	154	128
5000	d=2	935	465	315	232	182	156	129
10000	d=2	981	476	319	234	184	158	130
20000	d=2	1005	481	321	236	184	158	130
50000	d=2	1021	484	323	237	185	159	130
100	d=3	103	105	108	111	111	107	100
200	d=3	205	211	204	180	157	144	126
300	d=3	308	300	253	210	177	159	137
400	d=3	410	354	281	228	189	167	143
500	d=3	513	392	302	239	197	173	146
700	d=3	665	439	325	253	205	179	151
1000	d=3	799	481	346	264	211	185	155
2000	d=3	999	538	373	279	221	191	159
5000	d=3	1155	577	390	289	226	195	161
10000	d=3	1215	591	397	291	228	196	162
20000	d=3	1247	598	400	293	229	196	162
50000	d=3	1267	602	401	294	230	198	163
100	d=4	103	105	108	111	115	116	112
200	d=4	205	211	216	203	180	166	148
300	d=4	308	316	286	242	207	186	161
400	d=4	410	396	324	266	222	196	168
500	d=4	513	446	350	280	231	204	173
700	d=4	716	509	382	298	241	212	179
1000	d=4	906	562	408	312	251	219	183
2000	d=4	1165	634	441	331	262	226	189
5000	d=4	1362	683	463	342	269	232	191
10000	d=4	1438	700	470	347	271	233	193
20000	d=4	1478	709	475	348	272	234	194
50000	d=4	1504	715	477	350	272	234	194

D.4: Sensitivity Relative to the Controllers At 80%

Negatives Examined Annually	Acceptance Number	Positivity Rate 20.0%	23.0%	25.0%	28.0%	30.0%	33.0%	35.0%
		Total Sample Required						
100	d=0	36	34	32	29	27	25	23
200	d=0	43	38	36	32	30	27	26
300	d=0	45	40	37	33	31	28	26
400	d=0	46	40	37	33	31	28	26
500	d=0	48	42	39	35	31	28	26
700	d=0	49	42	39	35	31	28	26
1000	d=0	49	43	40	35	33	28	28
2000	d=0	50	43	40	35	33	30	28
5000	d=0	50	44	40	36	33	30	28
10000	d=0	51	44	40	36	33	30	28
20000	d=0	51	44	40	36	33	30	28
50000	d=0	51	44	40	36	33	30	28
100	d=1	60	55	52	49	46	42	40
200	d=1	71	64	60	54	50	46	43
300	d=1	75	66	63	56	53	48	45
400	d=1	78	69	64	57	53	48	46
500	d=1	79	70	65	58	54	49	46
700	d=1	81	71	65	58	54	49	46
1000	d=1	83	71	67	60	56	49	46
2000	d=1	84	73	68	60	56	51	48
5000	d=1	85	74	68	61	56	51	48
10000	d=1	86	74	68	61	56	51	48
20000	d=1	86	74	69	61	56	51	48
50000	d=1	86	74	69	61	57	51	48
100	d=2	79	73	69	64	61	57	54
200	d=2	95	86	80	72	69	63	58
300	d=2	101	90	84	76	71	64	62
400	d=2	105	92	87	78	73	66	62
500	d=2	108	95	88	79	73	67	63
700	d=2	110	96	89	79	74	67	63
1000	d=2	111	97	91	81	76	69	63
2000	d=2	114	100	92	82	76	69	65
5000	d=2	116	100	93	82	77	69	65
10000	d=2	116	101	93	83	77	69	65
20000	d=2	116	101	93	83	77	70	65
50000	d=2	116	101	93	83	77	70	65
100	d=3	95	88	84	78	74	70	66
200	d=3	116	105	99	90	84	78	74
300	d=3	125	112	104	94	89	81	75
400	d=3	130	114	107	96	90	82	77
500	d=3	133	117	109	97	91	84	78
700	d=3	136	119	111	100	93	84	78
1000	d=3	139	122	113	100	94	85	80
2000	d=3	143	123	115	103	96	87	80
5000	d=3	144	126	116	103	96	87	82
10000	d=3	145	126	116	103	96	87	82
20000	d=3	145	126	117	104	96	87	82
50000	d=3	145	126	117	104	96	87	82
100	d=4	108	101	97	92	87	82	78
200	d=4	136	123	116	106	100	93	88
300	d=4	148	131	123	111	104	96	91
400	d=4	153	136	127	114	107	97	92
500	d=4	156	139	129	117	109	99	92
700	d=4	161	142	132	118	110	100	94
1000	d=4	165	144	135	119	111	101	95
2000	d=4	169	147	136	122	113	103	95
5000	d=4	171	149	139	122	114	103	97
10000	d=4	173	149	139	124	114	103	97
20000	d=4	173	151	139	124	114	103	97
50000	d=4	173	151	139	124	114	103	97

D.4: Sensitivity Relative to the Controllers At 85%

		Positivity Rate						
		2.5%	5.0%	7.5%	10.0%	13.0%	15.0%	18.0%
Negatives Examined Annually	**Acceptance Number**	**Total Sample Required**						
100	d=0	87	80	71	64	59	55	50
200	d=0	151	126	106	89	78	71	62
300	d=0	198	158	126	103	87	79	67
400	d=0	236	180	141	111	94	82	71
500	d=0	267	197	150	117	98	86	72
700	d=0	313	221	162	124	102	89	74
1000	d=0	360	242	174	131	107	93	77
2000	d=0	436	274	189	139	111	96	79
5000	d=0	499	297	199	144	115	99	80
10000	d=0	524	305	203	146	116	99	82
20000	d=0	538	309	204	147	116	100	82
50000	d=0	547	313	205	148	117	100	82
100	d=1	103	105	105	98	92	87	79
200	d=1	205	192	166	143	126	115	101
300	d=1	293	247	203	167	144	128	111
400	d=1	361	287	227	182	154	136	117
500	d=1	415	317	244	192	161	142	121
700	d=1	496	358	266	206	170	148	124
1000	d=1	579	396	285	217	177	154	128
2000	d=1	713	451	312	230	185	160	133
5000	d=1	824	491	331	240	192	165	135
10000	d=1	868	506	337	243	193	166	137
20000	d=1	892	514	341	244	194	167	137
50000	d=1	907	519	343	246	195	167	137
100	d=2	103	105	108	112	111	107	101
200	d=2	205	211	204	182	163	151	133
300	d=2	308	301	259	218	189	171	148
400	d=2	410	362	294	239	205	182	156
500	d=2	498	405	319	254	214	189	161
700	d=2	624	466	352	273	226	199	168
1000	d=2	747	522	381	289	237	207	173
2000	d=2	942	601	418	310	249	216	179
5000	d=2	1102	660	444	323	259	222	183
10000	d=2	1166	681	454	328	261	225	184
20000	d=2	1201	693	459	330	263	226	185
50000	d=2	1223	700	462	332	263	226	185
100	d=3	103	105	108	111	115	118	116
200	d=3	205	211	216	210	193	180	161
300	d=3	308	316	301	260	229	207	180
400	d=3	410	411	350	289	248	222	191
500	d=3	513	474	384	310	262	233	199
700	d=3	705	558	428	334	279	246	207
1000	d=3	879	633	466	356	293	255	215
2000	d=3	1145	739	517	383	310	268	222
5000	d=3	1359	817	551	401	321	276	228
10000	d=3	1443	845	564	408	325	279	229
20000	d=3	1489	860	570	411	326	280	230
50000	d=3	1518	868	574	412	328	281	230
100	d=4	103	105	108	111	115	118	122
200	d=4	205	211	216	222	216	205	187
300	d=4	308	316	324	294	263	240	211
400	d=4	410	421	395	333	290	260	226
500	d=4	513	519	440	360	307	273	234
700	d=4	718	635	497	392	328	289	245
1000	d=4	978	733	546	419	345	302	254
2000	d=4	1329	868	610	453	367	318	263
5000	d=4	1601	965	653	476	380	328	271
10000	d=4	1708	1001	669	483	386	332	273
20000	d=4	1765	1020	677	488	389	333	274
50000	d=4	1800	1032	682	490	390	334	274

D.4: Sensitivity Relative to the Controllers At 85%

Negatives Examined Annually	Acceptance Number	Positivity Rate 20.0%	23.0%	25.0%	28.0%	30.0%	33.0%	35.0%
		Total Sample Required						
100	d=0	48	43	41	38	36	34	32
200	d=0	58	52	48	44	41	37	35
300	d=0	63	55	51	46	43	40	37
400	d=0	65	57	53	47	44	40	38
500	d=0	66	58	53	49	44	40	38
700	d=0	69	60	55	49	46	42	38
1000	d=0	70	61	56	50	46	42	40
2000	d=0	73	62	57	50	47	43	40
5000	d=0	74	62	57	51	47	43	40
10000	d=0	74	64	57	51	47	43	40
20000	d=0	74	64	59	51	47	43	40
50000	d=0	74	64	59	51	47	43	40
100	d=1	76	70	67	63	60	57	54
200	d=1	95	86	80	74	69	64	62
300	d=1	104	91	85	78	73	67	63
400	d=1	108	95	88	79	74	69	65
500	d=1	111	97	91	81	76	69	66
700	d=1	114	100	92	82	77	70	66
1000	d=1	118	101	93	83	79	72	68
2000	d=1	121	104	96	85	80	72	68
5000	d=1	123	105	97	86	80	73	69
10000	d=1	124	106	97	86	80	73	69
20000	d=1	124	106	97	86	81	73	69
50000	d=1	124	106	99	88	81	73	69
100	d=2	98	91	88	82	79	75	72
200	d=2	125	113	107	97	93	87	82
300	d=2	138	122	115	104	97	90	86
400	d=2	144	127	119	107	100	93	88
500	d=2	149	130	121	110	103	94	89
700	d=2	154	134	124	111	104	96	91
1000	d=2	158	138	127	114	106	97	91
2000	d=2	164	142	131	115	109	99	92
5000	d=2	166	143	132	117	109	99	94
10000	d=2	168	144	132	118	110	100	94
20000	d=2	168	144	133	118	110	100	94
50000	d=2	169	144	133	118	110	100	94
100	d=3	114	108	105	100	96	91	88
200	d=3	153	138	131	119	114	106	102
300	d=3	169	151	141	128	121	112	106
400	d=3	178	157	147	132	124	115	109
500	d=3	184	161	151	135	127	116	111
700	d=3	190	166	155	139	130	119	112
1000	d=3	196	170	157	142	131	121	114
2000	d=3	203	175	161	144	134	122	115
5000	d=3	208	178	164	146	136	124	117
10000	d=3	209	179	165	147	137	124	117
20000	d=3	209	181	165	147	137	124	117
50000	d=3	210	181	165	147	137	124	117
100	d=4	124	122	119	114	110	106	103
200	d=4	176	161	152	140	134	125	120
300	d=4	198	177	165	151	143	133	126
400	d=4	209	186	173	157	147	136	129
500	d=4	216	191	177	160	150	139	131
700	d=4	225	197	183	164	154	142	134
1000	d=4	233	203	187	168	157	143	135
2000	d=4	240	208	192	171	160	146	137
5000	d=4	246	212	196	174	161	148	138
10000	d=4	248	213	196	175	163	148	140
20000	d=4	249	214	197	175	163	148	140
50000	d=4	249	214	197	175	163	148	140

D.4: Sensitivity Relative to the Controllers At 90%

		Positivity Rate						
		2.5%	5.0%	7.5%	10.0%	13.0%	15.0%	18.0%
Negatives Examined Annually	**Acceptance Number**	**Total Sample Required**						
100	d=0	93	86	82	78	71	68	65
200	d=0	168	146	130	118	102	94	88
300	d=0	232	189	162	143	120	109	99
400	d=0	285	223	185	160	130	118	106
500	d=0	330	249	202	172	138	124	111
700	d=0	404	288	227	189	148	132	117
1000	d=0	486	326	249	203	156	139	122
2000	d=0	637	386	281	223	168	147	128
5000	d=0	783	434	305	238	175	153	132
10000	d=0	847	453	314	242	178	154	133
20000	d=0	883	462	318	246	179	155	134
50000	d=0	907	468	321	247	179	156	134
100	d=1	103	105	108	110	107	104	101
200	d=1	205	208	197	183	162	152	141
300	d=1	307	287	254	228	192	176	162
400	d=1	406	346	295	257	211	193	174
500	d=1	489	393	325	279	225	204	183
700	d=1	622	461	368	308	243	218	193
1000	d=1	766	528	406	333	257	229	201
2000	d=1	1030	633	463	369	277	244	212
5000	d=1	1285	716	504	394	291	254	220
10000	d=1	1397	748	520	403	295	258	222
20000	d=1	1461	766	528	408	298	259	223
50000	d=1	1502	777	533	410	299	260	224
100	d=2	103	105	108	111	115	119	121
200	d=2	205	211	216	219	202	193	182
300	d=2	308	316	309	287	248	231	212
400	d=2	410	414	372	331	277	253	230
500	d=2	513	485	416	362	297	269	243
700	d=2	717	588	479	406	322	289	257
1000	d=2	946	687	536	442	344	306	271
2000	d=2	1339	839	617	494	372	328	287
5000	d=2	1710	960	678	530	392	342	296
10000	d=2	1872	1006	699	543	399	347	300
20000	d=2	1964	1032	711	550	402	349	302
50000	d=2	2023	1047	719	554	405	352	304
100	d=3	103	105	108	111	115	118	122
200	d=3	205	211	216	222	228	222	213
300	d=3	308	316	324	324	293	275	255
400	d=3	410	421	422	389	332	306	279
500	d=3	513	526	486	432	360	328	296
700	d=3	718	682	573	490	394	354	317
1000	d=3	1026	820	650	541	423	376	333
2000	d=3	1597	1025	759	609	461	406	355
5000	d=3	2095	1185	839	657	486	425	368
10000	d=3	2311	1247	868	674	495	432	373
20000	d=3	2432	1280	883	683	500	435	376
50000	d=3	2510	1300	892	689	502	436	377
100	d=4	103	105	108	111	115	118	122
200	d=4	205	211	216	222	230	235	237
300	d=4	308	316	324	333	328	312	293
400	d=4	410	421	432	430	380	353	324
500	d=4	513	526	533	491	415	381	346
700	d=4	718	735	652	567	460	415	372
1000	d=4	1026	929	752	631	497	444	393
2000	d=4	1811	1196	892	718	545	480	420
5000	d=4	2454	1398	991	778	576	504	437
10000	d=4	2727	1476	1028	799	587	512	443
20000	d=4	2879	1517	1048	810	593	516	446
50000	d=4	2975	1543	1059	817	597	519	448

D.4: Sensitivity Relative to the Controllers At 90%

Negatives Examined Annually	Acceptance Number	Positivity Rate 20.0%	23.0%	25.0%	28.0%	30.0%	33.0%	35.0%
		Total Sample Required						
100	d=0	61	58	56	53	51	48	46
200	d=0	81	74	71	65	61	57	55
300	d=0	90	82	77	71	66	61	58
400	d=0	96	87	81	74	69	63	60
500	d=0	100	90	83	75	70	64	62
700	d=0	104	94	87	78	71	66	63
1000	d=0	108	96	88	79	73	67	63
2000	d=0	113	100	92	82	76	69	65
5000	d=0	116	103	93	83	77	70	66
10000	d=0	118	103	93	83	77	70	66
20000	d=0	118	103	95	85	77	70	66
50000	d=0	118	104	95	85	77	70	66
100	d=1	96	92	89	86	83	79	77
200	d=1	131	122	115	107	101	96	91
300	d=1	148	135	127	117	110	101	97
400	d=1	158	143	133	122	114	106	100
500	d=1	165	148	137	125	117	107	102
700	d=1	173	155	143	129	120	110	105
1000	d=1	180	160	148	133	123	112	106
2000	d=1	188	166	153	138	127	115	109
5000	d=1	194	170	156	140	129	116	111
10000	d=1	195	171	157	140	130	118	111
20000	d=1	196	173	157	142	130	118	111
50000	d=1	196	173	159	142	130	118	111
100	d=2	119	116	113	110	106	103	100
200	d=2	170	160	152	142	134	127	122
300	d=2	195	179	168	156	146	136	131
400	d=2	210	191	179	164	153	142	135
500	d=2	219	199	185	168	157	145	138
700	d=2	231	208	192	175	163	149	142
1000	d=2	241	216	199	179	166	152	145
2000	d=2	254	225	207	185	171	157	148
5000	d=2	261	230	211	189	174	158	149
10000	d=2	264	232	213	190	176	160	151
20000	d=2	265	234	213	192	176	160	151
50000	d=2	266	234	215	192	176	160	151
100	d=3	125	130	129	128	126	122	120
200	d=3	203	191	183	172	163	154	149
300	d=3	236	218	205	190	180	167	160
400	d=3	256	234	219	201	189	175	166
500	d=3	269	244	228	208	194	179	171
700	d=3	285	256	237	217	201	185	175
1000	d=3	298	266	245	222	206	190	178
2000	d=3	314	279	256	231	213	194	183
5000	d=3	325	287	263	235	217	197	186
10000	d=3	328	290	264	238	219	199	188
20000	d=3	330	291	265	238	219	199	188
50000	d=3	331	291	267	239	219	200	188
100	d=4	125	130	133	139	139	137	135
200	d=4	229	218	209	199	190	181	174
300	d=4	273	253	240	224	210	197	189
400	d=4	299	274	257	236	221	206	197
500	d=4	315	287	268	244	229	212	202
700	d=4	335	303	280	256	237	219	208
1000	d=4	351	314	291	264	244	224	212
2000	d=4	373	330	304	274	253	231	218
5000	d=4	385	340	312	279	257	234	222
10000	d=4	390	343	315	282	259	236	223
20000	d=4	391	345	316	283	260	237	223
50000	d=4	394	347	316	283	260	237	223

D.5: Blinded Rechecking of Sputum Smear Examinations for Acid-Fast Bacilli

Peripheral Laboratory: _____

Local technician(s): _____

Date sampled: _____

Period in lab. register checked: _____

Second level technician: _____

Laboratory: _____

Third level technician: _____

Laboratory: _____

Peripheral results	Final countercheck results					
	Negative	1-9 afb	1+	2+	3+	Total
Negative						
1-9 afb/100 fields						
1+						
2+						
3+						
Total						

Summary of errors identified (nos.)				
Major Errors		Minor Errors		
HFP	HFN	LFP	LFN	QE
Total Major Errors:		Total Minor Errors:		

HFP = High False Positive; HFN = High False Negative; LFP = Low False Positive; LFN = Low False Negative; QE = Quantification Error

Goal Met Yes ___ No ___

Recommendations: _____

Peripheral lab		Results of		Spec	Size	Thick	Stain	Comment
Slide no.	Result	second level	third level					

Peripheral lab		Results of		Spec	Size	Thick	Stain	Comment
Slide no.	Result	second level	third level					

Spec, Size, Thickness and Stain: M= Marginal, P= Poor

D.5: Blinded Rechecking of Sputum Smear Examinations for Acid-Fast Bacilli

Peripheral lab		Results of		Spec	Size	Thick	Stain	Comment
Slide no.	Result	second level	third level					

Peripheral lab		Results of		Spec	Size	Thick	Stain	Comment
Slide no.	Result	second level	third level					

D.6: Rechecking Report of Multiple Laboratories for District Supervisor & NTP

District: _____

District Supervisor: _____

Sampling Period: _____

Supervising Laboratory: _____

Peripheral Lab	Annual Volume	SPR	# Slides Rechecked	HFN	HFP	LFN	LFP	QE	Total Errors
District Averages									

SPR :slide positivity rate HFN: High False Negatives HFP: High False Positives LFN: Now False Negatives
LFP: Low False Positives QE: Quantitation Errors

E1: Investigation of Errors

Pattern of errors	Possible causes	Suggested Investigation Steps
HFP and HFN	1. Unusable microscope	1. Examine a 3+ using that microscope
	2. Staining problems	2. Check stains and staining procedure
	3. Technician cannot recognize AFB	3. Test with clear-cut pos. / neg. and good microscope
	4. Gross neglect	4. Exclude other causes
A single HFP	1. Administrative error	1. Compare lab-register with QC-listing: correct slide number & result?
	2. As for more frequent HFP	2. Exclude causes of more frequent HFP
Regularly a HFP with or without LFP	1. Poor registration routine	1. Check accuracy of lab-register and other record keeping
	2. Staining problems/Fading	2. Check stains and staining procedure, consider restaining for rechecking
	3. Technician unclear on AFB appearance	3. Look for inconsistent results of suspects (regularly single pos. / low positive) in lab register
Rare LFP	To be expected	No investigation unless numbers increase
Many LFP, with or without occasional HFP	1. Problem with controllers	1. Evaluate controllers
	2. Technician unclear on AFB appearance	2. Recheck special sample of LFP from laboratory register
	3. Contaminated stain reagents	3. Test stain with known negative smears
Single HFN	1. Administrative error	1. Compare lab-register with QC-listing: correct slide number & result?
	2. Very thick smears and/or poor light	2. Evaluate quality of smear preparation, check microscope
	3. Gross neglect	3. Exclude other causes
Frequent HFN and/or Many LFN	1. Staining problems/Fading	1.Check stains and staining procedure, consider restaining for rechecking
	2. Poor smearing-technique	2. As above, single HFN
	3. Problems with microscope	3.Check microscope with positive slide
	4. Careless microscopy	4. Exclude other causes
	5. Contaminated stain reagents/water	5. Test stain with known negative smears
Very high proportion LFN	Contaminated meth. blue or rinse water	As above
Many QE (too low gradings)	1. Poor staining	As above
	2. Problems with microscope	As above