Revised National Tuberculosis Control Programme Laboratory Network

Guidelines for Quality Assurance of smear microscopy for diagnosing tuberculosis

Central TB Division, Directorate General of Health Services,
Ministry of Health & Family Welfare, Nirman Bhavan, New Delhi - 110011

National Tuberculosis Institute, Bangalore; Tuberculosis Research Centre, Chennai & World Health Organization, New Delhi, contributed in developing these guidelines

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1. INTRODUCTION

Much of the scientific foundation for the internationally recommended Directly Observed Treatment Short course chemotherapy strategy, "DOTS", was established in India. Since 1993, the Revised National Tuberculosis Control Programme (RNTCP), utilizing the DOTS strategy, is being implemented in India. By March 2006, RNTCP had expanded to cover over a billion populations. To date, entire country is fully covered under RNTCP. Expansion has entailed the establishment of over 12000 microscopy centers and the training of thousands of laboratory personnel and over 2400 laboratory supervisors.

During this rapid expansion, overall performance of the programme has remained good in terms of achieving the stated goals of the DOTS strategy. Cure and treatment success rates have consistently been above 85% and there has been a 7-fold reduction in TB deaths under RNTCP. Since its inception, the programme has initiated over 9.4 million patients on treatment, thus saving nearly 1.7 million additional lives.

Since RNTCP relies on sputum smear microscopy for diagnosis, categorization of patients and assessment of treatment progress, the credibility, success and sustainability of the programme depends on the strength of the laboratory network. The establishment of a well functioning laboratory network that provides the population with easy access to high quality smear microscopy services is of the highest priority for RNTCP. Poor quality microscopy services have serious implications for the programme, including the failure to detect persons with infectious TB who will continue to spread infection in the community, or leading to unnecessary treatment for "non-cases." Errors in the reading of follow up smears may result in patients being placed on prolonged treatment, or in treatment being discontinued prematurely.

An effective quality assurance (QA) system of the RNTCP, sputum smear microscopy network is of crucial importance for the future of the programme. QA is a total system consisting of internal quality control (QC), assessment of performance using external quality assessment (EQA) methods, and continuous quality improvement (QI) of laboratory services. QA of laboratory services is a complex issue, highly dependent on the available resources in the respective country or state, structure of the health system and laboratory network, and incidence of disease. To optimize QA, decentralization of the supervision and monitoring of the laboratory network is essential, and capacity building of the states to undertake these activities becomes a priority. This process requires the active support and participation of the respective administrative levels in the country. The definitions of QC, EQA and QI are explained below.

Quality Control (QC) Also called Internal Quality Assurance, includes all means by which the laboratory personnel performing TB smear microscopy control the process, including checking of instrument, new lots of staining solutions, smear preparation, grading etc. It is 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 evaluation of entire process of smear microscopy, and random blinded re-checking of routine 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 of patient slides, using both un-blinded and blinded procedures. EQA is also termed "proficiency testing' as described by IUATLD.

Quality Improvement (QI) A process by which all components of smear microscopy diagnostic services are carefully analysed with the aim of looking for ways to permanently remove obstacles to success. Appropriate data collection, data analysis, correct interpretation of the results 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 mostly relies on effective on-site evaluation visits.

EQA activities at each level of Laboratory Network

2. LABORATORY NETWORK

To provide TB smear microscopy services with an easy access for the entire population, a network of RNTCP designated microscopy centres (DMCs) with competency in acid-fast sputum smear microscopy, has been established. Each DMC caters to an approximate population of 1,00,000. The network of DMCs is supported by larger regional laboratories (Intermediate Reference Laboratories or IRLs), and overseen by a fewer National TB Reference Laboratories (NTI, TRC, LRS and JALMA). The higher centres must have the capacity to plan and implement quality assurance activities in a well-organized fashion, and be capable of ensuring action that leads to improved quality and performance of the DMC network. In view of the vast area of the country, for the purposes of RNTCP's QA programme, the laboratory network is typically organized according to three levels of general health service under the RNTCP.

a. National level (National to State);
b. State level (State to District); and
c. TB Unit level (TU to DMC).

The revised network for quality assurance at each level is discussed under three external quality assessment (EQA) activities to evaluate laboratory performance:

- On-site Evaluation
- Panel Testing
- Random Blinded Rechecking

2.a On-Site Evaluation

A field visit is an ideal way to obtain a realistic assessment of the conditions and skills practiced in the laboratory. On-site evaluation of IRLs and DTC/DMCs is therefore an essential component of a meaningful QA programme.

Ideally, on-site evaluation should be performed at least once a year by personnel from a higher-level laboratory (IRL/NRL) in order to evaluate the overall operational conditions in the microscopy centers. 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. 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.

- At least once a month visit by STLS to the DMC, is required.
- At least once a year visit by laboratory supervisors is recommended for IRLs by NRLs and for District TB Centres (DTCs) by IRLs.
- When poor performance has been identified through on-site evaluation, blinded rechecking or panel testing, additional visits by trained laboratory personnel from a higher level laboratory (the IRL or NRL laboratory Supervisor) are mandatory to perform a comprehensive evaluation of all laboratory procedures, implement corrective action, and provide training.

The visit includes a comprehensive assessment of laboratory safety including infection control measures; 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.

The RNTCP has established a system to monitor laboratory practices based on the IUATLD and WHO guidelines. The NRLs provide training to all IRL personnel responsible for on-site evaluation. Additionally, non-laboratory personnel (e.g., DTOs) should acquire working knowledge of routine laboratory operations, including proper RNTCP procedures, appropriate supplies, laboratory safety, basic microscope operations, and requirements of panel testing or rechecking programmes operated by the RNTCP. Laboratory Supervisors 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 wherever needed.

Infection control (IC) measures

Increased risk of hospital transmission has been documented in a variety of settings. A variety of factors contribute to hospital transmission. The greatest risk of transmission occurs when smear positive tuberculosis patients remain undiagnosed and untreated. The key, therefore, to the reduction of hospital risk is early diagnosis and prompt initiation of treatment of TB cases.

The first priority in infection control is the use of administrative control measures to prevent the generation of infectious droplet nuclei, thereby reducing the exposure of the health care workers (HCWs) and patients to *M. tuberculosis* bacilli. Measures include patient education, correct sputum collection, achievement of early detection and high cure rate among smear positive pulmonary tuberculosis patients, strict adherence to laboratory SOPs, and adequate training of HCWs to implement the IC plan.

An IC plan should include: strict and correct implementation of RNTCP diagnostic and treatment guidelines in the facility; identification of risk areas in the respective facility and area-specific infection control recommendations; improvement of the ventilation of the health care facility; education of the patients regarding cough hygiene; and assessment of HCW training needs and adequate training of the HCWs for implementation of the plan. It is essential that the administrative in-charge of the respective health care facility be assigned responsibility and accorded authority to monitor the implementation of the IC plan.

Proper waste disposal as per the established norms, water treatment, disinfections and sterilization of equipment, all can reduce the risk of exposure to infection of patients, health care providers and the community. All health care facilities should be kept clean, which will reduce infection by any virulent organism. Cleaning of premises and floors with water and detergent is recommended. Cleaning with a disinfectant is usually not necessary unless there is spillage with potentially infectious material. All HCWs working at the district level should receive ongoing education at least once a year regarding the basic concepts of *M. tuberculosis* transmission and pathogenesis, the signs and symptoms of TB, the increased risk of TB disease in persons with HIV infection, and other immunosuppressive conditions, who also are infected with *M. tuberculosis.*

Checklists

Checklists are required 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. An important component of using any checklist is to provide sufficient training and standardization so that the checklists are used consistently. Checklists may be refined to focus on problems that are frequently identified or most likely to occur, such as preparation of stains or errors in grading. Wherever the RNTCP Supervisory Register is being used by the visited health facility, a summary of findings and recommendations from the visit should be written in the register. This will provide written documentation of the visit and findings and will also assist subsequent evaluations to monitor improvements. Where the RNTCP Supervisory Register is yet to be introduced, copies of summary of the results of checklist should be left behind in the unit receiving the on-site evaluation

Monthly feedback should be given to the MO in-charge of the respective DMC by the DTO. Consolidated quarterly summaries of results will be sent by the DTO to the concerned CMO/DMO of the respective district and to the Intermediate Reference Laboratories (IRLs). The IRLs will submit a consolidated quarterly report to the concerned State TB Officer and National Reference Laboratory. In turn the NRL will submit a consolidated quarterly report to the Central TB Division. 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.

Comprehensive checklists for on-site evaluation of IRLs, DTCs and DMCs are provided in the annexures. These checklists contain 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. These detailed checklists provide a template that may be adapted to meet the specific needs of EQA at each level. 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.

Use of a simple standardized checklist by well-trained district supervisors, can reduce the time necessary to evaluate a laboratory, especially when supervisors are very familiar with the process. Any simple checklist will require 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 adequately trained laboratory or non-laboratory personnel should ensure the availability and practice of the following:

i) Written standard operating procedures (RNTCP Laboratory Manuals and Modules, display of charts on smear preparation, staining and reading etc)
ii) Adequate supply of reagents within expiry date.
iii) Proper, well functioning equipment and an adequate supply of consumables

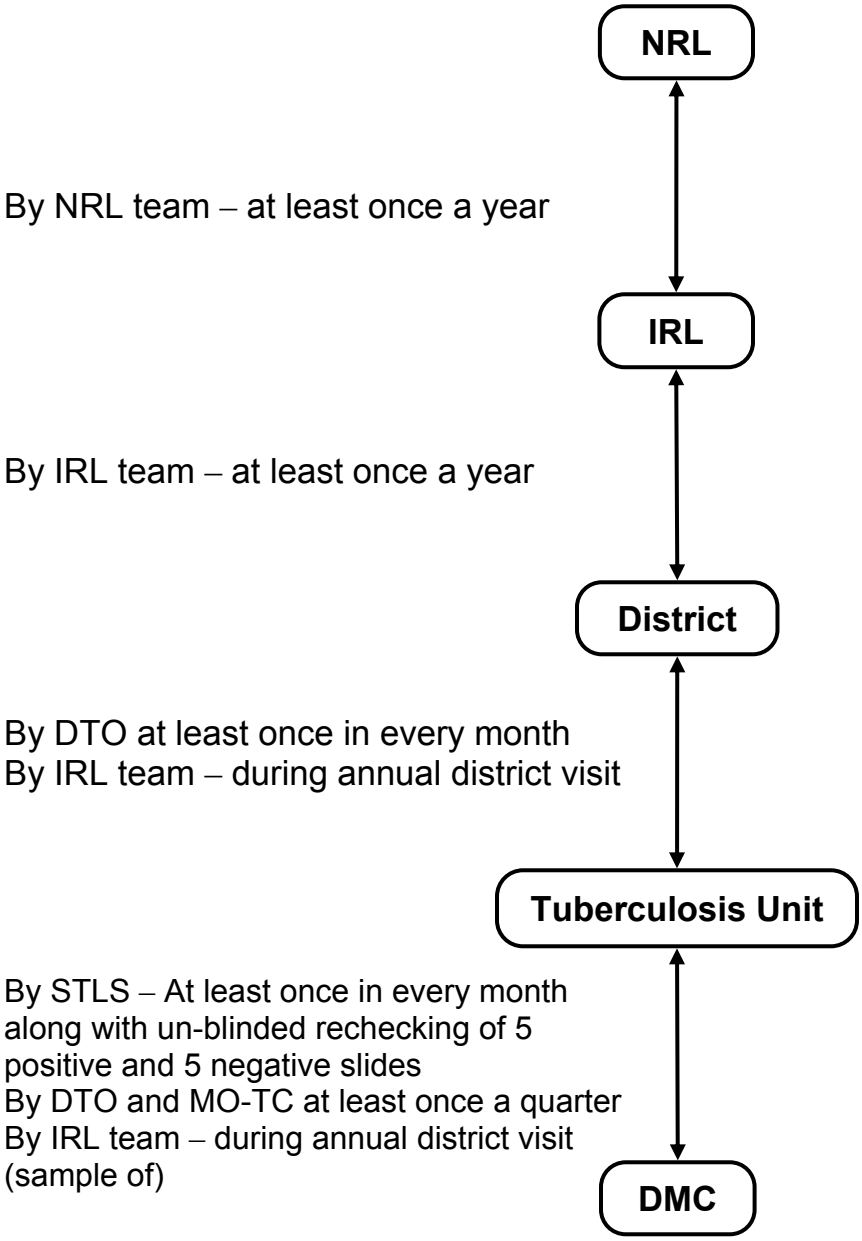
iv) Internal QC such, as use of unstained positive and negative smears for every new batch of stains / reagents - Assuring that positive and negative control slides are used with all newly made batches of stains.
v) Laboratory safety practices including infection control measures..
vi) Accurate record keeping consistent with the requirements of RNTCP (for example "triangulation" between laboratory register, TB register and treatment cards).
vii) Prompt reporting of results to treatment centers or physicians.
viii) Availability of a functional binocular microscope. At a minimum, district supervisors must be familiar with simple microscope function, and be able to visualize a clear image through the microscope lens. In addition, all States should have annual maintenance contracts for the binocular microscopes.
ix) Proper storage of patient's slides for EQA including rechecking to enable the supervisors to collect appropriate number of slides to be sent to reference laboratory.
x) Staff with adequate training with refresher courses with a capability of undertaking corrective action when appropriate.
xi) Evaluation of workload and proportion of positive smears to be examined.
xii) All chest symptomatics who are smear positive in the laboratory register are recorded in the TB register. Registers other than standard RNTCP registers are not to be maintained. Also laboratories should not do "pre-screening before testing" of cases.
xiii) The findings and need for corrective action or additional resources that are required.

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:

i) Evaluating sputum collection procedures.
ii) Observing and evaluating procedures for smear preparation, staining, and reading.
iii) Rechecking several positive and negative smears to evaluate staining, smear thickness, smear size, and results.
iv) 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 development of strategies and activities for improvement of quality.

On-site supervision



2.b Panel Testing

Panel testing is a method of EQA that is used to determine whether a laboratory technician can adequately perform AFB smear microscopy. This method evaluates individual performance in staining and reading, not as such all the laboratory activities. Utilization of panel testing for EQA is considered to be less effective than random blinded rechecking of routine slides because it does not monitor routine performance.

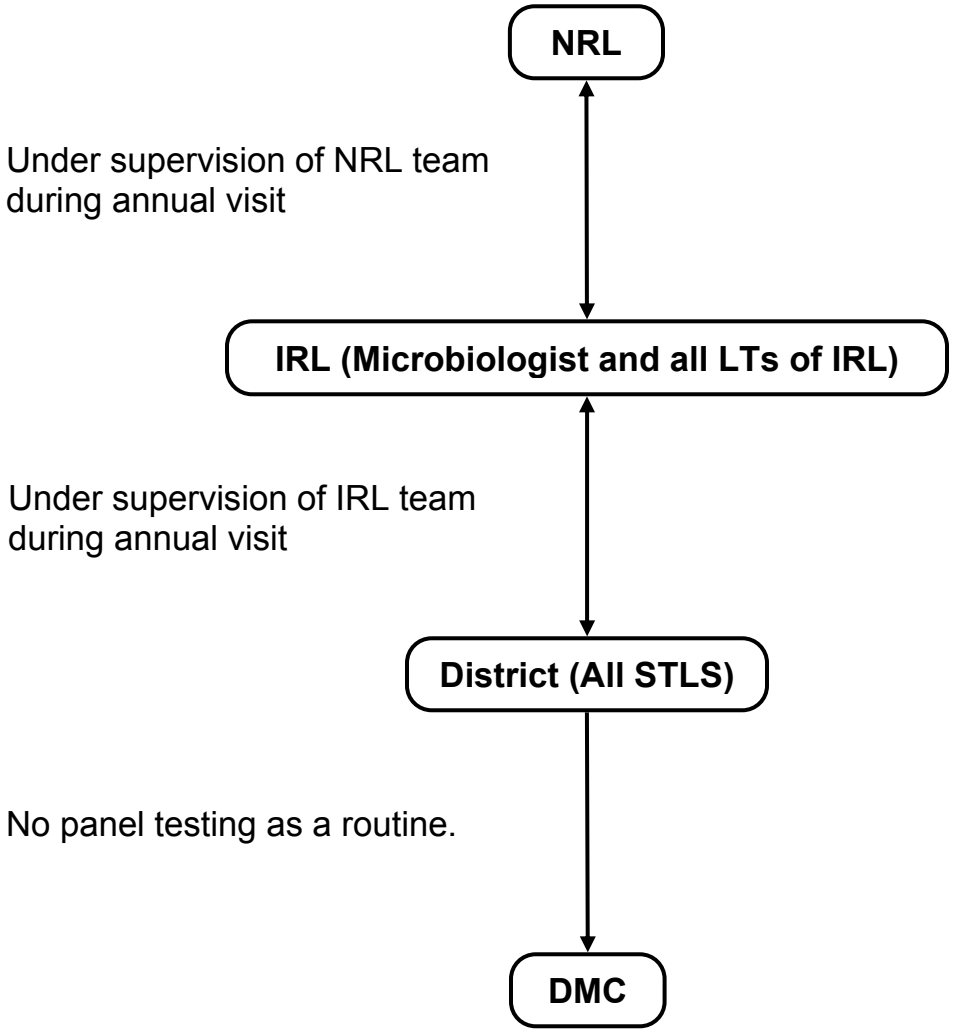
Panel testing is useful to:

- Supplement re-checking programmes.
- Provide information on the capabilities of the peripheral laboratories prior to implementing a re-checking program.
- Assess status level 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 re-checking program.
- A panel consists of a batch of stained and /or unstained smears that are sent out by the higher-level 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 be included (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 all laboratory technicians and microbiologists 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.

Panel testing under RNTCP is used for IRLs and DTCs during on-site evaluation, because these institutions do not have routine slides for blinded rechecking. Panel testing is not performed as a routine in DMCs, as they will have regular on-site evaluation and blinded rechecking.

The details of panel testing under RNTCP is given in the chapter for NRLs and IRLs.

Panel testing



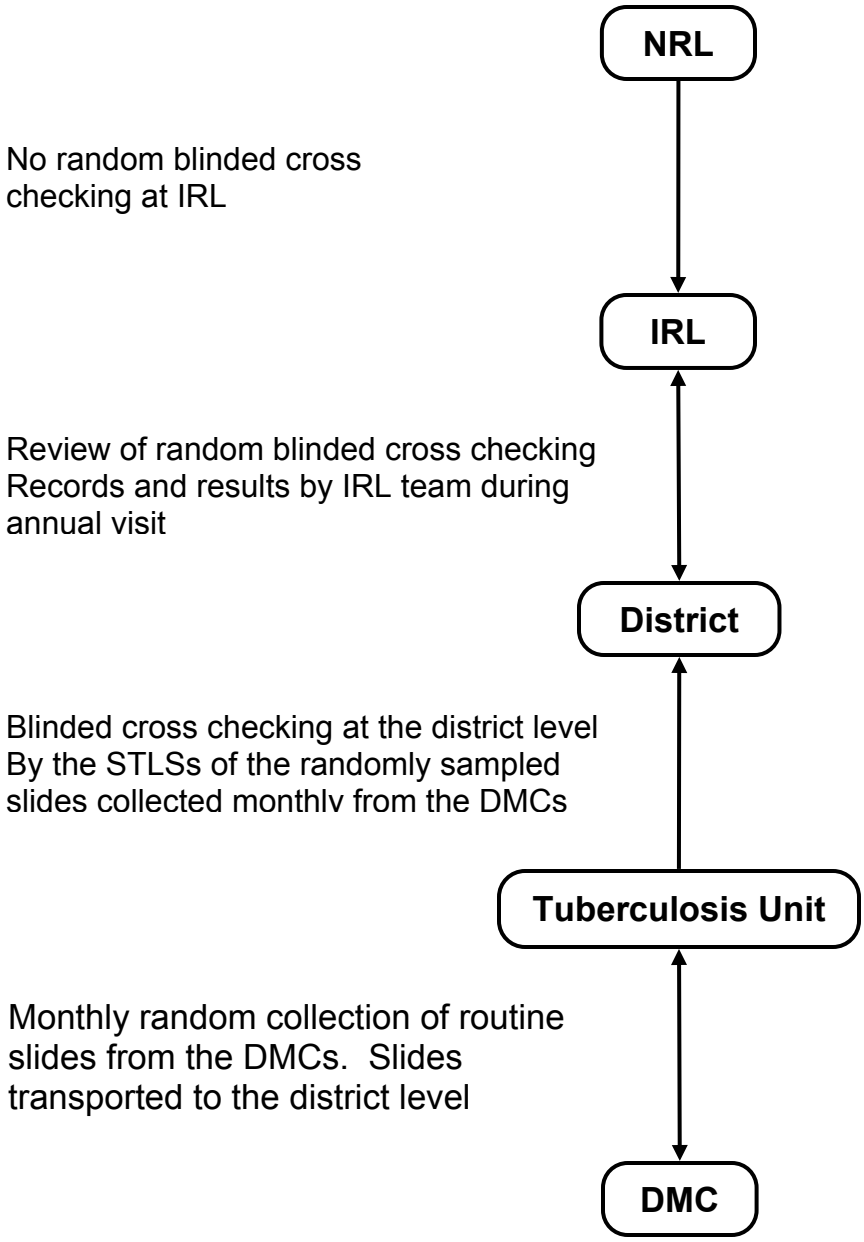
2.c Random Blinded Rechecking of Routine Slides

This EQA method provides reliable assurance that a district has an efficient AFB microscopy laboratory network supporting RNTCP. Blinded rechecking is a process of rereading a statistically valid 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 contains sufficient number of randomly selected slides to be representative.
- The supervisor of the laboratory (controller), must be unaware of the original result of peripheral laboratory technician to prevent bias, i.e. is "blinded".
- 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 timely feedback and improvements to the laboratories that are supervised.

Random blinded rechecking (RBRC) of routine slides from the DMCs is to be implemented throughout the RNTCP laboratory network. A system utilizing Lot Quality Assurance Sampling (LQAS) method has been pilot tested in a number of districts and found feasible for implementation in all DMCs, the details of which is given in the respective chapter of the guidelines.

Random blinded cross checking



2.1 NATIONAL REFERENCE LABORATORIES

The four National Reference Laboratories (NRLs) namely, National TB Institute (NTI), Bangalore, TB Research Centre (TRC), Chennai, Lala Ram Sarup Institute of TB and Respiratory Diseases (LRS), Delhi and JALMA, Agra are entrusted with the task of EQA of selected states each. The details of their role are given below.

2.1.a On-Site Evaluation

Technical supervisory visits offer the best opportunity to review activities in the respective IRL, identify potential sources of error, and implement corrective action. For this reason, on-site supervisory visits by experienced staff from the NRL are recommended at least once a year, and more frequently if significant problems are identified.

A team headed by a Microbiologist and at least one LT from NRL, will visit the IRL for about 3 days. The IRL Director and STO are to be present during the visit, and the IRL Director will ensure that the full complement of IRL staff is present during the on-site evaluation.

The visit includes a comprehensive assessment of laboratory safety including IC measures, 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 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. On-site visits by experienced laboratory personnel from a NRL provide an opportunity for immediate problem solving, corrective action and on-site retraining.

Detailed procedures have been developed under RNCTP to monitor laboratory practices. The NRL provides training to all personnel responsible for on-site evaluation. Non-laboratory personnel will obtain 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 programmes operated by the RNTCP. Laboratory personnel are made knowledgeable in all operational and technical elements of AFB smear microscopy and develop sufficient expertise to observe technicians performing routine tasks. They would also obtain training to facilitate quality improvement through on the spot problem solving and suggestions for corrective action when needed.

When very poor performance has been identified through panel testing or rechecking, and/or the reasons for the problems are not readily apparent or correctable through basic action, an expanded visit by NRL laboratory personnel to the IRL may be necessary.

Checklists
Checklists have been developed 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. An important component of using any checklist is to provide sufficient training and standardization so that the checklists are used consistently. The comprehensive list has all operational elements to be observed that 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. The comprehensive checklist for on-site evaluation is provided in Checklist NRL-OSE (page 44). Use of this checklist reduces the time necessary to evaluate a laboratory.

Results of checklists should always be sent from NRL to the respective STO / IRL Director and CTD after analysis. STO / IRL Director to give action-taken report to CTD within a month. Documentation of any significant problems is required for improvement of strategies and systems. The respective NRL will also submit to CTD a consolidated quarterly summary supervisory report based on the findings of the lower level's supervisory visit reports.

2.1.b Panel Testing

The use of a test panel of unstained slides has the advantage of testing several aspects of the designated state level LT's technical performance, including preparation of staining reagents, staining procedure, reading and reporting of results. The use of stained smears alone, will only assess reading capability, and will not provide information on the LT's capability to 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.

Preparation of Test Smears

There are two methods for preparation of the sets of panel testing smears: (i) use of manufactured smears; and (ii) the re-use of stained patient smears. RNTCP will use manufactured smears as well as patient-wise smears for panel testing. Manufactured panel smears and encouraged, where ever biosafety cabinets are not available, the IRLs can use the patient-wise unstained smears. The NRLs will use manufactured panel slides. With specimens of known positive and negative patients a collection of positive slides with a consistent, predetermined quantity of AFB per slide, as well as negative slides with authentic background material are prepared. By using manufactured slides, all laboratory technicians involved in panel testing will receive a standardized set of slides, which will minimize variation in expected results due to differences in smear consistency. The procedure for manufacture of the panel-testing smears is given in Annexure A (page 39).

Validation of the slides is required for ascertaining consistency of slides prior to sending out test panels. This is a pre-requisite to 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. To increase the efficiency of

manufacturing slides, NRLs should develop the capacity to produce and validate batches of 50-100 slides that can be stored for future use in preparing test panel sets.

Panel Testing Sets
Sets of slides with identical composition of positives and negatives can be made from the prepared batches of slides. A logbook of Test Slide Sets can be used to select slide sets and record the original batch numbers and expected results for a 5 slide panel testing exercise. This form can also be used to record and evaluate the results from one or more supervisors undergoing panel slides test.

Number and Type of Smears
A set of five slides, which represents about ¼ of the maximum slides that a technician can examine per working day without losing quality, is considered as an acceptable number. A panel consisting of 5 pre-fixed unstained manufactured slides per IRL technician and microbiologist, will be given to all the microbiologists and LTs of the respective IRL for processing, reading, and reporting of results during the on-site evaluation visit by the NRL team.

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. 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. There must be variation in the slide sets (number of positives and negatives) sent with each new panel testing exercise so that technicians do not expect the same composition of slides each time. The composition of the types of slides in the panel will be decided by the NRLs. A different set will be used for each LT and microbiologist at the IRL.

It is important that technicians be given sufficient time to read the test smears and that the time spent reading these slides be the same as they would routinely spend on patient smears. Since technicians may spend an excessive amount of time reading slides when they know they are being tested, the panel test of the IRL LT's will be conducted during the on-site evaluation visit of the NRL team and under the supervision of members of the visiting NRL team. An approximate time of 25 to 35 minutes for five slides, depending on the grade of smear, will be allowed for the panel test conducted. The results should be made available to NRL Team leader within the day. This will facilitate the rechecking of discordant results of panel testing slides and feedback to be given by the NRL team before their departure.

Forms for Test Laboratories to record results
Standardized forms for recording and reporting results must be provided for the panel test exercise. 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 reporting 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 Checklist NRL OSE (page 44).

Frequency of testing
Panel testing may 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 IRL. After this it will be conducted once a year during the annual evaluation visit to the IRL from the NRL level. Panel testing may also be used intermittently whenever it is felt necessary.

Evaluation and interpretation of results
Panel testing evaluates performance using the best of smears, and generally the technicians know they are being tested. Therefore, the best performance results when using this method may be expected. For reporting, both the number and the type of errors are considered. It is helpful to collate the results from other IRLs visited / technicians before reporting. If a majority of technicians fail to report correct results for the same slide, it may represent a problem with slide preparation at the NRL, the results should be excluded and an investigation conducted. A form for evaluating and reporting aggregate results is found in Checklist NRL-OSE (page 44).

Classification of Errors

Result of technician	Result of controller				
	Negative	1-9 AFB/ 100 fields	1+	2+	3+
Negative	Correct	LFN	HFN	HFN	HFN
1-9 AFB/ 100 fields	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

Reporting System
The report form is shown in Checklist NRL-OSE (page 44).

Feedback
Reports should include both individual results, as well as aggregate performance for all laboratories / technicians tested. Reports are to be sent to the STO, IRL, and CTD and discussed with the concerned IRL lab personnel. Reports should include comments on performance, possible sources of error and suggestions or requirements for remedial action.

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 NRL. For individual LTs, investigation should include on-site evaluation to determine the source of the problem.

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 Annexure K. 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 / technicians tested. If it is determined that the consistency and quality of the slides used in the panel testing exercise was acceptable, and a majority of laboratories / technicians submit unacceptable results, this represents serious problems in AFB microscopy and a need for additional resources is indicated. Additional resources should be obtained for supervisory visits, correction of problems identified in individual laboratories or of individual technicians, including replacement of microscopes (and/or microscope objectives), retraining if needed, and follow up panel testing.

Resolving Discrepancies

No system for developing test panels and conducting the test at the IRL is completely without problems. This could include: i) technical difficulties in preparing individual slides; ii) error in the initial reading of a smear at the NRL; iii) incorrect recording of expected results; and iv) fading of stained smears during transport.

Therefore any panel testing system must include a mechanism to resolve discrepant results. If the panel test is conducted unsupervised by a higher level supervisor, then panel slides may require returning to the higher level laboratory for rereading, or sending a supervisor from the higher level laboratory to the peripheral site for comprehensive on-site evaluation and re-reading of discordant test panel slides with individual laboratory technicians. However, usually these sorts of problems are infrequent and can be resolved at a local level. Unsupervised panel reading is not recommended under RNTCP.

2.1.c Blinded rechecking

There is no random blinded rechecking of routine slides at the IRL level, as the IRL LTs are not expected to perform patient diagnosis or follow up microscopy functions.

2.2 INTERMEDIATE LEVEL REFERENCE LABORATORY

Each state will have its own designated Intermediate level Reference Laboratory (IRL), for the purposes of smear microscopy laboratory QA activities within the respective state. The State TB Training and Demonstration Centres (STDCs), are to be made functional to act as the IRLs. In those States where an STDC does not exist, a Public Heath Laboratory or Medical College Microbiology Department with available infrastructure, committed and accountable staff, will be identified to carry out the laboratory QA activities of RNTCP. The IRL will be monitored by the respective designated NRL and will undergo accreditation for sputum microscopy and mycobacterial culture and drug sensitivity testing by the NRL.

2.2.a On-site evaluation

Once a year visits to the DTC laboratories by the IRL laboratory team are required. More frequent visits are required in case of poor performance. The IRL team will consist of the IRL Microbiologist, MO, Laboratory supervisor or LT. The visit to DTC will be for a minimum of two days. The DTO and all STLSs of the district will be present at the site of visit. If required, the team may also visit any TU and/or DMC in the district where deficiencies have been documented by the district staff.

On-site evaluation by non-laboratory personnel is generally limited to assuring that RNTCP 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 including IC measures. IRL laboratory supervisors will ensure that standard operating procedures (SOP) are in place, internal QC is performed, and a functional microscope is available at DTC laboratory. Sufficient time must be allotted for the visit to include observation of all the work associated with AFB smear microscopy, including 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.

On-site visits by experienced laboratory personnel from an IRL provide an opportunity for immediate problem solving, corrective action and on-site retraining. They provide an opportunity for reviewing the results of the supervisory visits made by STLS during the year and the offering of suggestions if required. In addition, they are useful to verify data on TB laboratory workload, positivity rate for suspects and follow up examinations being done at MCs of the district.

Some of the aspects that need to be checked by the IRL personnel during their on-site evaluation include availability of weighing machine, measuring cylinders, distilled water, chemicals of known potency, flasks, and details of batch of reagents prepared and the results of internal quality controls for each batch maintained by STLS etc. Knowledge of STLS in infection control measures at DMCs' may also be assessed.

A more comprehensive review of laboratory conditions and practices may be necessary when poor performance is identified during the yearly supervisory visit, or through panel testing and reasons for the poor performance are not readily apparent or correctable through more basic corrective actions.

Checklist
RNTCP has developed checklists to assist both the IRL laboratory and non-laboratory supervisors during the field visit and to allow for the collection and analysis of standard data for subsequent remedial action. The team will report on a standard checklist (Checklist IRL-OSE, page 53). A report will be submitted by the team to the DTO and CMO of the district visited. The report will also be sent to STO and the IRL Director. A summary of the report, and not a copy of the complete checklist, will be sent to the concerned NRL. The DTO or CMO of the district is to submit an action taken report on the team's recommendations to the STO within a month of the IRL visit.

2.2.b Panel Testing

The basic principles and methodology for panel testing to be used by IRL are as those recommended for the NRLs. The salient points of panel testing by the IRL are given below.

Preparation of test smears
Only manufactured smears are to be used for the panel testing of the STLSs. The IRL LTs will be trained in the manufacturing procedure at the NRLs. Subsequently the manufacture and validation of slides panel testing of districts will be done at the IRL. The necessary infrastructure, such as bio-safety cabinets and centrifuges, Vortex mixer, etc., will be procured. The same validation procedure as at the NRLs is to be followed at the IRLs.

A panel of 5 slides per STLS in the district, will be used, comprising of manufactured unstained pre-fixed slides. Composition of slides will be decided by the IRL and will be known only to the Microbiologist / MO of the team. The test panel will include slides with different grades of positivity in order to evaluate the ability of the technicians to correctly grade positive slides. In the initial phase of implementation, the respective NRL will guide the IRL in choosing the composition of panel sets to be used. All STLS of the district being visited will be evaluated. A different panel will be used for each STLS.

The panel testing slides of each STLS of the district will be conducted during the on-site evaluation visit by the IRL team under the supervision of the IRL team members. An approximate time of 5 to 15 minutes per slide, depending on the grade of smear, will be allowed for the panel test conducted. The results should be made available to IRL Team leader within the day. This will facilitate the rechecking of discordant results of panel testing slides and feedback to be given by the IRL team before their departure (Checklist IRL-OSE.

Standardized forms for recording and reporting results must be provided for the panel test exercise. This will help to reduce confusion regarding the expectations and requirements of the exercise. Each STLS must complete the test panel independently, and not as part of a group effort. It is important to instruct the STLSs **NOT** to share results, since this is being used to evaluate the performance of each individual STLS. Each STLS must complete a form with his or her own results. A reporting 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 Checklist IRL-OSE

Frequency of testing
Panel testing may be done as a one time, initial exercise in the early stages of EQA to obtain baseline data on capabilities of STLS in the respective districts. After this it will be conducted once a year during the annual evaluation visit to the DTC from the IRL level. Panel testing may also be used intermittently whenever it is felt necessary.

Evaluation and Interpretation of Results

The reporting system used will be as for the panel testing at the IRL level. Reporting will be on a standard format developed for that purpose and it will be a part of the on-site evaluation format (Checklist IRL-OSE).

Feedback
Feedback, investigation of errors and corrective action to be taken are as described for the panel testing at the IRL level. Reports are to be sent by IRL to the DTO, STO, and NRL. Compiled report for whole State is to be sent to CTD by the IRL. Reports should include description of errors, possible reasons/ sources of error and suggestions or requirements for remedial action.

Resolving Discrepancies
As for the testing at the IRL level, the process for panel testing at the district level has included on-site evaluation and re-reading of discordant test panel slides with individual STLSs.

2.2.c Blinded rechecking

During the annual IRL on-site evaluation visit to the DTC, the IRL team will review the whole year's blinded rechecking records and results from the district, identify deficiencies and recommend corrective actions that need to be taken. They will ensure that the blinding procedures are being strictly followed by all involved in the process.

2.3 TB UNIT

The sub-district level, TB Unit (TU) level, is responsible for a number of designated microscopy centres (DMC), which are supervised by personnel from TU. DMCs cater to a population of approximately one lakh (1,00,000) in normal circumstances and one per 50,000 population in tribal and other special circumstances, and they should ensure that all persons with cough for 3 weeks or more in their catchment areas undergo three sputum smear examinations. Policies pertaining to technical issues are given in the RNTCP Laboratory Manual. Training to laboratory technicians (LT) is given using the RNTCP LT training module designed for them. The LT should perform smear microscopy as per the technical and operational guidelines of RNTCP. The results are communicated to the treating physicians who in turn should follow the recommended procedure for managing tuberculosis disease, until cure / completion of treatment as the case may be. Documentation and reporting should be as per RNTCP and these are facilitated by the team of supervisory staff at the TU.

One TU is established for every five lakhs (5,00,000) population. The Senior TB Laboratory Supervisor (STLS) makes supervisory visits to all DMCs at least once a month. The STLS provides support, guidance, and trouble-shooting to ensure quality sputum microscopy services. The STLS is also responsible for quality control of laboratory work in all the DMCs under his administrative control, which are normally five in number. The STLS should not be employed in providing routine microscopy services as it will compromise their supervisory role.

2.3.a On-Site Evaluation

Monthly visits to the DMCs by the district / sub-district supervisors (STLS) are required. On-site evaluation by non-laboratory personnel i.e. the DTO who is administratively and technically in charge of the STLS, is generally limited in assuring the following RNTCP requirements: recording and reporting of results; assessing operational conditions, safety, supplies, equipment and total workload. Regular visits by the district supervisor also provide an opportunity to review the STLSs checklist and written recommendations

Supervisors should make sure that Standard Operating Procedures (SOP) are in place and SOPs are displayed in all DMCs, internal QC as per RNTCP is performed, and a functional binocular microscope is available. Since the ability to identify AFB and report the same is considered essential for anyone working in TB control programme where diagnosis and follow-up are largely based on AFB-microscopy, reading 5 positive and 5 negative smears is necessary as part of the routine monthly visit. Visits by STLS are also useful to collect data on TB laboratory workload, smear positivity rate for suspects and follow up examinations. These are important for several reasons. Heavy workload may contribute to poor performance. A low workload may not be adequate to maintain proficiency in reading AFB smears. At DMCs, where smear microscopy is only a part of LTs activity, a workload of less than 10 a week or more than 20-25 AFB smears a day may interfere with good quality of smear microscopy.

Monitoring slide positivity rates is necessary to determine appropriate sample sizes for a random blinded rechecking programme. Any significant changes in the indicators may indicate performance problems and for calculation of necessary laboratory supplies. 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 posted.

The visit includes a comprehensive assessment of laboratory safety including IC measures; 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. Some of the activities at DMCs that need to be observed during supervision visit include proper disinfection of all specimens using disinfectants such as 5% Phenol, or 5% phenolic solution, availability of reagents within expiry date etc. Care should be taken to see that newly prepared batch of reagents should not be mixed with old batch of reagents.

As part of on-site evaluation, the visiting STLS will review in an unblinded manner 5 positive and 5 negative slides selected systematically from the RNTCP TB Laboratory Register during each visit to the DMC. The slides selected for un-blinded crosschecking are for the period between last and the current visit of STLS. Systematic selection is stipulated so that, as far as possible, each of the slides checked are from a different patient. The STLS should indicate the date of current visit by drawing a line on the left margin of the lab register, below the row with last lab entry. Results of cross checking should be recorded in the laboratory register. The method of selection is similar to that indicated for random blinded re-checking (RBRC), except that the selection is made separately for positive and negative slides. The pencil marking on the Laboratory register for selected slides should be 'X', so as to differentiate the selection made for RBRC. Results of un-blinded cross checking should be recorded in the remarks column of the RNTCP laboratory register. Also the STLS will record the results in the supervisory report (Checklist TU-OSE, page 63).

On-site visits by STLS provide an opportunity for immediate problem solving, corrective action and on-site retraining.

Checklist

Checklists have been developed to assist both laboratory and non-laboratory supervisors during the field visit and to allow for the collection and analysis of data for subsequent remedial action. An important component of using any checklist is to provide sufficient training and standardization so that the checklists are used consistently (Checklist TU-OSE).

The checklist has to be used by STLS and a shorter version by DTO/MOTC during each on-site evaluation visit to DMC. As indicated in the previous chapters, 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. Once the supervisor has identified actual response to a question, s/he converts the same into simple, objective "Yes/No" evaluation criteria, yielding data that can easily be entered into a database for long-term tracking and comparing performance.

For example, the following illustration may be seen to assess the process under Item No. 26 of the checklist (on page 64) i.e., "Control smears are used for each new batch of stain".

- Open ended question to be asked and answers elicited or verified in documents:
    - What is the maximum volume of any reagent that can be prepared in the laboratory?
        - Answer: 5 liters (i.e., one batch)
    - What is the volume of reagents prepared at one time point, how many batches and what were the batch numbers?
        - 15 liters or three batchs prepared in a particular day, each of '1% carbol fuchsin' (10^{th}, 11^{th} and 12^{th} batch for the year), for 25% Sulphuric acid (15^{th}, 16^{th} and 17^{th} batch for the year) and 0.1% Methylene blue (10^{th}, 11^{th} and 12^{th} batch for the year)
    - How are QC slides prepared, marked and stored?
        - Twenty smears were prepared and heat fixed from a patient with 3+ results in all three specimens and marked with glass marking diamond as 'QC-P'. Twenty smears were prepared from a patient with three negative specimens with plenty of pus cells and marked with glass marking diamond as 'QC-N'. They were stored in a separate slide box marked 'QC Slides of TU xxx'.
    - How are QC slides used?
        - A set consisting of each of these is used for new batch of reagents for QC testing at DTC lab.
        - A set consisting of each of these is also supplied to DMCs along with new batch of reagents for QC testing at DMCs.
    - What is the combination of each batch of reagents tested at DTC lab with quality control (QC) slides?
        - One known 1+ positive and negative unstained slides were tested for 10^{th} batch of 1% carbol fuchsin + 15^{th} batch of 25% Sulphuric acid + 10^{th} batch of 0.1% Methylene blue.
    - Where were the entries made for this activity?
        - Entries were made in the separate register (and should also be verified by the supervisors before answering).
    - What is the result of this QC?

        - 1+ positive was found to be 1+ and negative slide was found to be negative
    - What were the QC results for other two batches of reagents?
        - No other QC tests were done

- What is the response by the supervisor in the column for 'Adequate / acceptable' for the question No.26 and why?
    - 'N' because the other two batches have not been QC tested before supplying to DMCs, though s/he has adapted a very good method for maintaining records.
- What would be entry to be made in the column 'remark' for the question No. 26?
    - The person preparing the reagents should test each batch of reagents with 1+ positive and negative QC slides, even if they were prepared the same day?

It may note that only open ended questions such as 'what / why / where / which / how' have been used by the supervisors to analyze the process of QC, leading identification of error and an ultimate response of 'no' for this question. This has also resulted in suggestion of proper corrective action. Close ended questions such as 'do / did / is / whether' would not elicit this type of correct response and hence are not encouraged to be used.

The supervisory format (Checklist TU-OSE, page 63) must be completed at each visit. The STLS should discuss the findings and corrective actions with LT and obtain the signature of MO of DMC on the checklist. S/he should immediately enter the summary of 'action required' in the Supervision Register before leaving the DMC. STLS should submit the summary report of DMCs under him to DTO on a monthly basis. Separate files should be maintained by STLS for checklists including summary reports of each DMC in respective TU and submit them for review by higher level supervisors including on-site evaluation visits by the IRL to the district.

Summary of results of checklists for each DMC in the district should sent by DTO once every quarter for analysis by the respective CMO/DMO and IRL, and a state level consolidated summary will be prepared by the respective IRL every quarter from the district summaries for submission to STO and NRL. 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.

The checklist for on-site evaluation is provided in Checklist TU-OSE (page 63). This is a detailed checklist, which 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. The checklist is to be printed by the DTO and made available to all STLSs of the district.

On-site evaluation of the technical practices in the laboratory performed by properly trained STLS includes all of the operational elements listed under On-site evaluation in general as well as the following: (i) sputum collection procedures, (ii) procedures for smear preparation, staining, and reading, (iii) ensuring examination of positive and negative control slides with all newly made batches of stains, (iv) rechecking several positive and negative smears to evaluate staining, smear thickness, smear size, and results, (v) reviewing results of rechecking and (vi) providing suggestions for corrective action or implementing corrective action as needed.

Documentation of any significant problems is necessary to formulate plans with the DTO, STLS, MO, and LT to improve the quality of smear microscopy. As an interim measure, major findings of the supervision visit should be recorded as part of the monthly abstract in the RNTCP Laboratory Register. Format for monthly abstract is given in **Annexure M**, which will be written in the last few pages of Lab register**.** Both supervisor and laboratory technician should sign and record date on this list of findings. On subsequent visits, action taken in regard to the finding of previous visit should be recorded and signed by the supervisor and laboratory technician. Future versions of the Laboratory Register would contain separate pages for the recording of findings and action taken.

The on-site evaluation at DMC level is meant for immediate corrective action, and the analysis must be done at the TU and DTC level. Analyzed data (pooled as a summary) only is to be sent to IRL, STO and CTD. Immediate feedback is provided on-site by the STLS to the LT and MO in-charge of the respective DMC through the RNTCP Supervision Register and by the DTO within one month. If immediate feedback is not used then this exercise will not serve any purpose. The MO in-charge of the DMC is to submit an action-taken report to the respective CMO within a month and the DTO to the STO / IRL Director along with their consolidated quarterly supervisory finding report. In addition, MO of the DMC will review annually the summaries of monthly abstract entered at the end of the respective Laboratory register.

2.3.b Panel Testing

Panel testing is not recommended for routine performance at the DMC level. Panel testing is to be used for re-training purposes under training conditions (e.g., on-site visit of IRL to DTC).

Unstained slides (one 1+ positive slide and one negative slide) should be used for internal quality control by STLSs to test every new batch of stains prepared. These slides are to be prepared by the STLS from available sputa samples. They should have distinct labels to facilitate their identification as Internal Quality Control slides.

2.3.c Random blinded rechecking of routine slides

Random 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:

1. A sufficient number, as per table on page 29, of randomly selected slides from the laboratory is chosen and forms a sample representative of the performance.

2. LT of DMC properly stores all slides until samples are collected by STLS.

3. The controller must be "blinded" from knowing the initial test results when rechecking the slides to prevent bias. Breaches in blinding protocol should be met with disciplinary action.

4. 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 easy implementation and sustainability of rechecking programmes.

5. Discrepant results are resolved by a second controller.

6. There must be a system to provide continual feedback and improvements to the laboratories that are supervised. Feedback should be provided within one month of on-site evaluation visit. Strong and consistent support is provided by RNTCP to implement and sustain functional rechecking programs. When combined with effective regular on-site evaluation, random blinded rechecking provides reliable assurance that RNTCP has an effective AFB microscopy laboratory network.

7. Performance is assessed based on the number and type of errors exceeding a predetermined threshold, rather than calculating a percentage of errors. Rechecking programmes are intended to assess overall laboratory performance, not to confirm any individual patient's diagnosis. Therefore, the emphasis on rechecking every positive slide has been 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.

8. The sampling method 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 has statistical validity that the laboratory is meeting performance expectations. If one or more errors are detected, the STLS 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. However, given the performance profile of smear microscopy, discrepancies (i.e., "errors") are expected and if they are consistently not found then the sampling procedure and/or blinding process should be re-examined.

9. Determining sample size. 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 as defined by RNTCP. The sample size is based on a modified statistical sampling method called **Lot Quality Assurance Sampling** (LQAS). Conceptually LQAS acts like a filter to separate acceptable laboratory standards from unacceptable quality. LQAS uses the smallest required numbers of negative smears for rechecking to indicate that a selected parameter (namely the number of False Negatives) has not been exceeded in the sample with 95% confidence limit, provided that after checking the sample size, not more than the specified number of the parameter `d' is found. If more than these numbers are observed**,** investigation of the possible causes should be undertaken at the supervisory level. Corrective action will be determined by the magnitude of the error and will be taken by the DTO/ CMO of the district. Existing reports have been revised as part of the new EQA protocol after being pilot tested.

   i) Under the LQAS method, sample size depends on total number of negatives slides processed each year, the positivity rate and expected performance (sensitivity) compared to the controllers.

   ii) Slide Positivity Rate (**SPR**): This is the proportion of positive smears among all slides (pretreatment and follow up) 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 DMC, TU, district, state, or country.

   iii) **SPR** is calculated by dividing the total number of positive slides by total number of slides and multiplying the resultant number by 100. The following is an illustration of how to calculate the SPR. DMCs with less than 5% SPR should be intensively assessed and efforts to minimize false negative results or improper referral of TB suspects, should be undertaken (Annexure L).

DMCs in the TU are listed with the following information: (a) number of all slides examined per year, (b) number of positive slides per year and (c) number of negative slides per year

Table 1
Calculation of SPR in each DMC

DMC	**Slides / year**	**Pos / year**	**Neg / year**	**SPR** = Pos slides / total slides x 100
A	1500	200	1300	**13.3%**
B	2550	351	2199	**13.8%**
C	1990	156	1834	**7.8%**
D	1006	72	934	**7.2%**
E	2005	141	1864	**7.0%**
Total	9051	920	8131	**10.2%**

For example, the SPR for DMC "D" is 7.2%.

iv) Annual Negative Slide Volume (ANSV) I the annual slide volume minus the number positive slides per year per centre. All health institutions performing AFB smear microscopy under RNTCP i.e. all RNTCP DMCs, should be included. For example, in DMC "D", ANSV is 934. Any DMC with ANSV of less than 500 should be evaluated and all efforts to improve ANSV should be undertaken. Possible reasons would be inadequate referral of TB suspects by MOs, non-involvement of private sector etc (Annexure L). In some areas, more centres than recommended under RNTCP may have been allowed to function as microscopy centres. which are likely to reduce the workload and quality of smear microscopy at DMCs.

v) Sensitivity: This is the expected performance in detecting positives, as compared to the controllers. The sensitivity, as defined here, is the detection of all positives, including low positives (1-9 AFB / 100). An overall sensitivity of 75-85% is recommended in the initial stages. **For RNTCP, 80% sensitivity has been selected**. Acceptable sensitivity for the subsequent years of EQA implementation will be determined by the RNTCP based on results from the field.

vi) Specificity: If specificity is 100% then no false positive is detected. For this example, 100% specificity is assumed, which is the proportion selected under RNTCP for this parameter.

vii) Acceptance number 'd' or 'critical value' (maximum number of errors allowed before action is taken) is taken as 0. The value of '0' has been selected for 'd' under RNTCP.

viii) The number of slides to be selected (annual sample size) will be decided beforehand using the table 2 given in page 29. It is based on SPR, Annual Negative slides and Sensitivity.

ix) ANSV and SPR for new DMCs can be obtained from the data available from their NTP lab register or annualized, if less than one year RNTCP data is available. For DMCs without any data for estimating ANSV and SPR, district averages are calculated and applied for the first year.

x) Determining sample size is not left to the STLS collecting the slides or to the technicians. See below the table that uses the criteria of 80% sensitivity, 100% specificity, and 0 acceptance number.

Table 2
Recommended Annual Sample Size[1]
(80% sensitivity, 100% Specificity and '0' Acceptance number)

Number of negative slides in the DMC in a year	Slide positivity rate (SPR%)				
	2.5-4.9	5.0[2]-7.49	7.5-9.9	10-14.9	≥15
	Annual sample size of both positive and negative slides (Monthly sample size[3] in parenthesis)				
301[4]-500	243 (21)	154 (13)	114 (10)	89 (8)	62 (6)
501-1000	318 (27)	180 (15)	128 (11)	96 (8)	66 (6)
>1000	456 (38)	216 (18)	144 (12)	104 (9)	69 (6)

[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.

[2] DMCs with less than 5% SPR should analyze the reasons for the same and should undertake the necessary corrective actions.

[3] The monthly sample size has been rounded off to the next higher number and annually adds up to equal or more than the annual sample size.

[4] The status of DMCs with Annual negative slide volume (ANSV) of ≤300 should be reassessed. If they can not be improved then they should be discontinued as DMCs. Till their status is finalized, those DMCs with ANSV less than 301 will use the sample size for 301-500 ANSV as applicable for the respective SPR range. If the ANSV is less than the indicated Annual Sample Size (ASS), the respective DMCs should submit all their slides for blinded re-checking. *For example, a DMC with ANSV <243 & SPR <4.9%, or ANSV <154 & SPR 5.0-7.5%, or ANSV <114 & SPR 7.49-9.9%, or ANSV <89 & SPR 10-14.9%, or ANSV <62 & SPR ≥15% should submit all slides for blinded re-checking.*

xi) In the example on page 28, the annual total negative slide volume (ANSV) for DMC "D" was 934 and SPR 7.2%. From the above annual sample size table, the column with the range of SPR ("5-7.49%") and the row with the number of ANSV of 501-1000 slides / year would be selected. Thus in the above example, the annual sample size would be 180 slides for DMC 'D', or a monthly sample of 15 slides to be collected by the STLS during their monthly supervision visit.

10. Slide Storage. The sampling system under LQAS method eliminates the need to select positive slides separately from negative slides; **therefore there is no need to store positive and negative slides separately.** The laboratory must store slides in a way that allows for easy retrieval of every slide identified for the rechecking sample. Therefore all slides are to be stored in the provided slide boxes in the same order as they are listed in the laboratory register. Slides are marked as 'a' and 'b' along with the lab serial number for first spot and early morning specimen. In order to maintain consistency with the laboratory register, one blank spaces should be left behind the first slide from a suspect patient so that the second slide can be added after it is read. One blank space should be left behind the first slide from a follow-up patient, so that the second slide can be added after it is read. Sufficient number of boxes must be available to preserve 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.

    Removal of immersion oil is to be done by placing the slides inverted with smear surface with immersion oil facing downwards, on tissue paper overnight, or till the immersion oil is completely absorbed. Care may be taken not to rub the slides on the tissue paper as this activity may remove the smear from slides. Store slides in boxes that do not allow the slides to touch each other (e.g., do not stack or press slides together). Always store slides in closed boxes away from direct sunlight.

11. Slide Collection. If the results of a rechecking programme 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 DMC must be blinded to the controllers. 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 also helps ensure blinding, since the whole sample will be naturally well mixed when the batch goes to the controller.

12. **Annual slide sample and the monthly sample for random blinded rechecking is to be determined by the DTO, assisted by the SA or DEO,**

based on LQAS method of sampling. Technical support for sampling will be provided to the DTO by the respective IRL based on previous year's data. Instructions will be sent by DTO to all STLS of the district, informing them of the total number of slides to be collected every month from each DMC. STLS then selects the required number of slides from the RNTCP Laboratory Register (Figure on page 33), and the LT records the results as per Annexure B. The slides collected for blinded rechecking should be from the previous calendar month. Annexure B is then put into an envelope and sealed. The number of slides packed is written on the top of the envelope. Both the slide box and the envelope must be clearly marked with the name of the respective DMC, the name of the TB Unit and district, and the month and year. The slide box and the sealed envelope are taken by the STLS for handing over to the DTO. The STLS should leave a corresponding number of empty slide boxes for the use of LT at DMC.

13. Slide Selection. To avoid bias, the technician in the DMC must never perform the selection or sampling of the slides. The STLS will collect the sample during their monthly visit. It is recommended that **one twelfth of the total annual sample size** be collected during the monthly visit of the STLS, as given in Table 2. In order to eliminate selection bias, slides are selected using the laboratory register. Once the STLS identifies which slides are to be collected on the collection form, the LT 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 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 have not understand the need to save slides for rechecking. The STLS should carefully consider the problem and provide criteria for corrective action. Based on the number of negative slides processed by the DMC, SPR, the annual sample size for blinded rechecking is determined. For example if the sample size is calculated to be 180 smears per year, 15 slides are to be collected during each monthly visit. If the STLS observes that the laboratory processed 82 slides since the last monthly visit, they could collect for example every fifth (82/15 =5.4 or 5th) slide randomly to obtain the required 15 slides. They may begin with any number between 1 to 5, say 3. The first random number may be selected by choosing last digit on any available currency note. The remaining slides are chosen by adding serially 5 till 15 slides are selected. In this example, the 3rd, 8th, 13th, 18th, 23rd, 28th, 33rd, 38th, 43rd, 48th, 53rd, 58th, 63rd, 68th and 73rd slides are selected to obtain 15 slides required for that month. Some training and direction on how to sample from the laboratory register for the STLSs is critical.

    The selected slides are marked off in the lab register, as shown in Figure 1. They are then taken out by STLS from their original slide box and arranged in a separate slide box marked as 'LQAS slides' with the name of DMC,

month, and year. The results of selected slides are entered by LT in Annex B, sealed, and handed over to STLS, who will **immediately** transfer the LQAS slide box and sealed cover to DTO.

It is preferable to collect the LQAS slides in first **week** of month in order to provide sufficient time for completion of re-checking process by the end of the month.

14. Blinded Rechecking Process: Blinded re-examination of selected slides is to be done by an STLS of another TU within the respective district. The STLS (controllers) must have demonstrated proficiency with the Ziehl-Neelsen staining and reading method (as seen by panel testing done by IRL). The same number of fields as specified in the RNTCP 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 versus saliva), appropriate size and thickness, and quality of staining (as per Annexure C). 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. It is crucial that the blinding process is strictly adhered to, otherwise the whole exercise will become invalid. **The DTO is responsible for ensuring that the blinding process is strictly followed.** All discrepant slides should be re-stained and re-examined by the second controller, as there is likelihood of fading of carbol fuchsin.

15. It is essential that the rechecking is performed in a blinded manner to ensure objectivity, with the first controller not knowing the initial result. However, the second controller (i.e. another STLS of the district or IRL supervisor) who is responsible for resolving discrepant results, must know both previous results. This should be done in a way that it is impossible for the second controller to determine which result was from the DMC LT and which was from the first 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 (e.g. 5 AFB / 300 fields). The reading is done after re-staining for all discordant slides. 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. This rechecking of discordant slides by a second controller also acts to evaluate the performance of the first controllers.

Figure 1

Method of selection of slides for Blinded Rechecking from TB Lab Register

Laboratory Register – Circles indicate slides to be collected

Lab Serial Number	Date	Name	Sex M/F	Address	Name of Treatment Unit	Reason for examination		Result of specimen		Signature	Remarks
						Diagnosis	Follow-up	**a**	**b**		
131	28/03/09					√		2+	1+		
NEW PAGE											
132	01/03/09					√		Neg			
133	01/03/09							Neg	1+		
134	02/03/09					RE		Neg	Neg		
135	02/03/09							1+			
136	02/03/09							Neg	Neg		
137	03/03/09						29/09	Neg			
138	03/03/09							Neg	6AFB		
139	04/03/09					√		1+	3AFB		
140	05/03/09					√		Neg	Neg		
141	05/03/09							Neg	Neg		
142	06/03/09							1+			
143	07/03/09					√		Neg	Neg		
144	07/03/09						32/09	Neg	Neg		
145	09/03/09							Neg			
146	09/03/09					√		Neg	Neg		
147	09/03/09							Neg	Neg		
148	10/03/09					√		Neg	Neg		
149	10/03/09							2+	1+		
150	11/03/09					√		Neg	Neg		
151	11/03/09							1+	Neg		
152	12/03/09					√		6AFB	2+		
153	12/03/09							Neg			
154	13/03/09					√		Neg	Neg		
155	14/03/09					√		Neg	Neg		
156	16/03/09						101/09	Neg	1+		
157	16/03/09							Neg	1+		
158	17/03/09					√		Neg	Neg		
159	18/03/09							2+	Neg		
160	18/03/09					√		Neg	Neg		
161	19/03/09					√		Neg	Neg		
162	20/03/09					RE		Neg	Neg		

Lab Serial Number	Date	Name	Sex M/F	Address	Name of Treatment Unit	Reason for examination		Result of specimen		Signature	Remarks
						Diagnosis	Follow-up	**a**	**b**		
163	21/03/09							Neg	1+		
164	21/03/09					√		Neg	Neg		
165	23/03/09					√		2+	3+		
166	24/03/09					√		Neg	Neg		
167	24/03/09							Neg	Neg		
168	25/03/09					√		Neg	Neg		
169	26/03/09					RE		Neg	Neg		
170	27/03/09							Neg			
171	28/03/09					√		Neg	Neg		
172	28/03/09					√		1+	Neg		
173	30/03/09					√		Neg	Neg		
174	31/03/09						51/09	Neg	Neg		
175	31/03/09					√		Neg	Neg		
176	31/03/09						124/09	Neg			
NEW PAGE											
177	01/04/05					RE		Neg	Neg		

16. The maximum number of slides that can be crosschecked by one STLS (first controller) in one day would be around 40. The manageable workload from EQA for an umpire reader would be a maximum of approximately 25 slides a day, as s/he is required to read more fields per discordant slide.

17. **Types of Errors** It is important to re-emphasize that random blinded **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 (Table 3), not on the potential impact of patient management.

Table 3
Classification of Errors

Result by DMC LT	Result of Controllers				
	Neg	1-9 AFB / 100 fields	1+	2+	3+
Neg	Correct	LFN	**HFN**	**HFN**	**HFN**
1-9 AFB/ 100 fields	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**

Performance is assessed based on the number and type of errors exceeding a predetermined threshold, rather than calculating a percentage of errors. In the table given on page 29, the acceptance number 'd' or 'critical value' (i.e. maximum number of errors allowed before action is taken) was taken as 0. 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. Since both major and minor errors are included in the calculation of the sample size, the interpretation of errors and the appropriate corrective action should depend on the number of and the type of errors, their evolution over time, and the resources available to implement corrective actions.

Logically, the rechecking will start by focusing on major errors and on laboratories with large numbers of errors. If errors are detected, the interpretation and appropriate action may be different depending on the number and type of error. High numbers of false positives should be a very rare occurrence. Clerical, administrative or sampling errors can lead to isolated HFP results. Higher rates of HFP are typically due to unusable microscopes or untrained or inexperienced LTs. An occasional HFN is to be expected due to the inherent limitations of smear microscopy. Higher rates are seen when the LTs are overworked, technical problems such as poor stains, insufficient staining time or heating, bad microscopes or inadequate training of LTs. As with false positives, higher numbers

of false negatives may indicate gross neglect and an overall lack of motivation on the part of the LT. Further details of investigation of errors are given in Annexure K.

Feedback

The primary purpose of the rechecking is to improve the overall quality of smear microscopy, therefore regular and timely feedback to the DMC is essential if any improvements in performance are to be expected. Although final analysis of the results and conclusions have to await completion of rechecking of the whole (i.e. annual) sample, preliminary observations, feedback and remedial action will often be possible at the end of each sampling period. Feedback will be given on a monthly basis to the respective DMCs using form in Annexure D. Feedback will include the return of slides with discordant results to be re-read by the original LT of the respective DMC. This will occur during the monthly on-site evaluation visit by the STLS responsible for the respective DMC. Potential sources of errors are to be investigated during these on-site evaluation visits. Appropriate corrective actions and/or remedial training are to be provided within one month.

Reporting

The Quarterly Programme Management Report (PMR) from the TU level will give details of the systematic un-blinded rechecking of 5 positive and 5 negative slides performed by the STLS during their monthly visits to the DMCs.

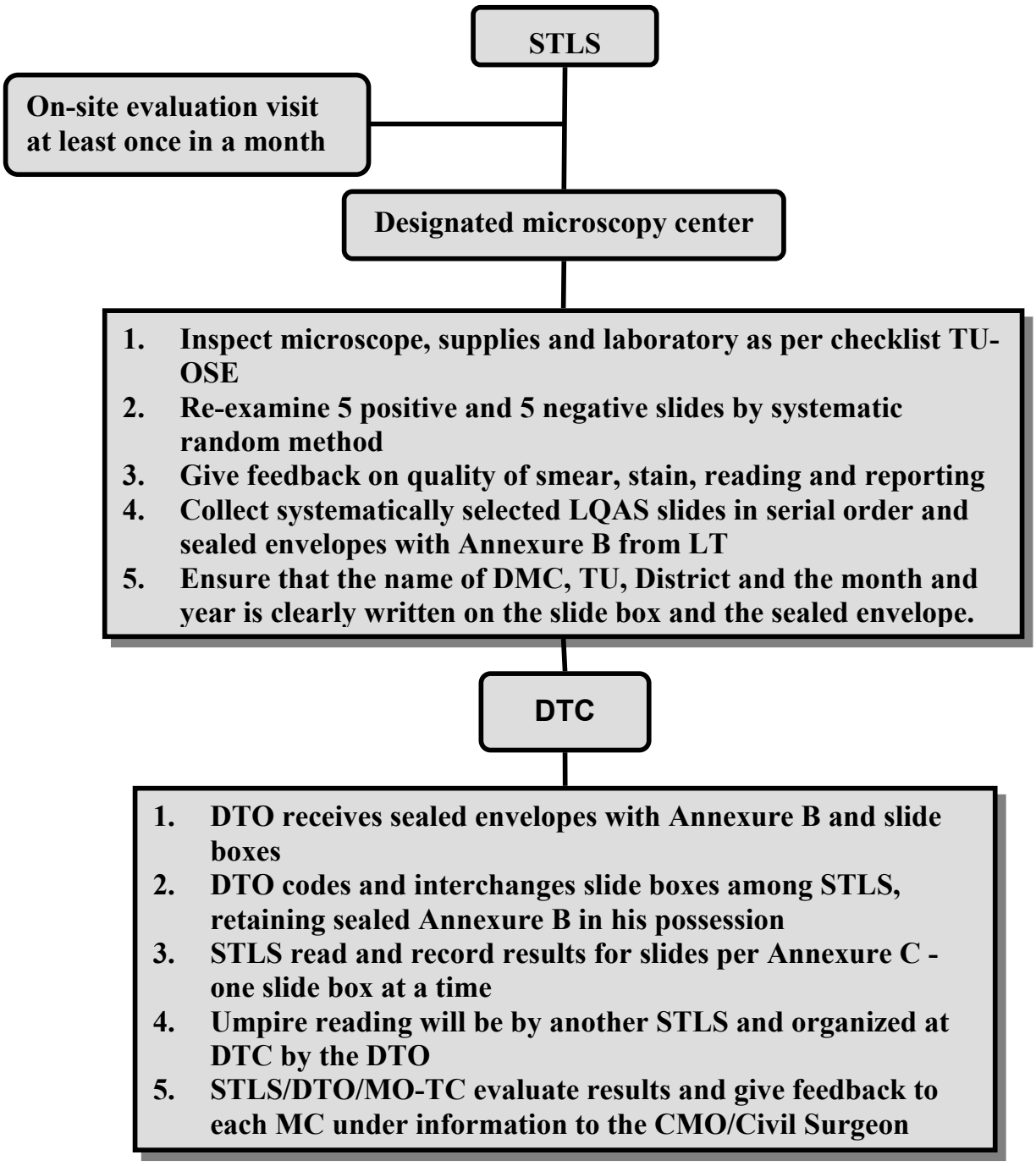
The District level PMR will report on the blinded rechecking activities, by DMC, performed at the DTC. Results reported will be on the RBRC activities of 2 quarters previously. For example when reporting in July 2005 i.e in Quarter 3 2005, the PMR report will include the results of the RBRC activities for the period January to March 2005 i.e. Quarter 1 2005. The reports submitted will be an interim cumulative set of results from the annual LQAS sample. The final annual report, for example for 2005, would be submitted during Quarter 2 2006.

District should send monthly and quarterly report of blinded rechecking as per Annexure E & F to IRL, and IRL will compile and send an annual report to NRL and CTD as per Annexure G.

The laboratory register will have a page earmarked for recording the monthly summary of reporting the (i) number of suspects examined and found positive, (ii) number of suspects subjected for repeat examinations performed and found positive, (iii) number patients subjected for follow-up examinations and found positive, (iv) number of slides examined (total, positive slides and negative slides) (Annexure M).

Note: In case of district having only one or two TUs, blinded rechecking will be conducted in collaboration with adjacent district.

QUALITY ASSURANCE NETWORK IN SPUTUM SMEAR MICROSCOPY – STLS ACTIVITY



Annexures, Appendices and Checklists

Annexure- A

Manufacturing procedure for panel test smears

This procedure is a self-explanatory laboratory method for producing multiple test slides from AFB positive and negative samples. The laboratory staff should read and understand the procedure and the testing protocols before developing test slides. This procedure has been reproduced / validated in state and national laboratories. If laboratory has difficulty in producing slides that meet the requirements for consistency they 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 NRL or IRL laboratory.
All procedures should be performed in a Biological Safety Cabinet.

Materials Required

50 ml plastic screw cap tubes
2% N-acetyl L-Cystine (NALC)
2.9% Sodium citrate.$2H_2O$
10% Formalin solution
Vortex mixer
Shaker
Hot air oven
Distilled water
Centrifuge
Slides

a. ***Collection of sputum specimens:*** Specimens from patients with known sputum smear results are collected. It is recommended that patients with all three smears positive for AFB with 3+ grade are selected for 'positive stock specimen'. Specimens from patients with all three smears showing plenty of pus cells and negative for AFB are selected for 'negative stock'. All specimens should be added with one drop of 10% Formalin immediately after collection to prevent further autolysis and are stored overnight at room temperature before being processed.

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

Amount: 3-4 ml or more; AFB load: 3+grade (>50 AFB/ field) by Ziehl-Neelsen direct smear; Color: Should be white to light green; avoid specimens which are blood stained; Thickness: Watery (less mucous) specimens are preferred to increase consistency.

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

Amount: 2 – 4 ml collected from ten to 12 different patients or multiple specimens from three or four patients; total requirement would be 30 ml or more; Color: white to green; Thickness: Watery (less mucous) specimens are preferred to increase consistency. An AFB negative specimen with 20 or more pus cells per field is preferred. Add one drop of 10% Formalin to each specimen of 3-4 ml to preserve pus cells. If facilities for processing large volumes of specimens are available, the negative specimens collected from different persons added with Formalin, can be mixed to form the negative stock.

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 shaken gently in a shaker at 50 rpm in incubator at 37^{o}C for about 10 minutes 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. ***Smears for AFB and pus cell counts of sputum suspensions/ dilutions:*** For assessing the number of AFB and pus cells, one drop of sputum suspension / dilution is spread on a slide with a smear size of 2 cm x 3 cm., dried & sterilized in a hot air oven at 60^{o}C for one hour without scorching.

Dilution Procedure

a. Using negative preparation as a diluent, dilutions are made according to RNTCP Guidelines for AFB quantification:

   0 AFB/100 fields: negative
   1-9 AFB/100 fields: 'Scanty' or exact number of AFB seen
   10-99 AFB/ minimum of 100 fields: 1+
   1-10 AFB/field in minimum of 50 fields: 2+
   >10 AFB/field in minimum of 20 fields: 3+

b. It is 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 "Scanty" smears.

c. Make 5 ml of each suspension in order 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 the volume of positive sputum to be added.
DC - is desired AFB concentration.
AC - is actual AFB concentration.
A - is the total volume of the required stock suspension.

Example:

Negative suspension

Volume of negative stock suspension (after liquefaction) = 20 ml

Calibrated drops per ml of suspension = 15 drops/ ml

Positive stock suspension

Volume of positive stock suspension (after liquefaction) = 6 ml

Calibrated drops per ml of suspension = 14 drops/ ml

AFB concentration in the stock suspension after liquefaction (AC) = 40 AFB/field

Suspension required

Grade of positive smear required = 3+

Final volume of 3+ suspension required = 5 ml. Approximately, 50 slides may be made from one ml of suspension. Some amount of 3+ suspension may be required for preparing 2+ suspension.

Hence, if about 200 slides have to be manufactured, about 4 ml of suspension is required and it is advised to prepare five ml or more of 3+ suspension.

Required concentration of AFB of 3+ suspension (DC) =20 AFB/field

Calculation for 3+ preparation of grade suspension

DC = 20, AC = 40, A= 5

N = (20 /40)*5

N = 2.5 ml or 14 x 2.5 = 35 drops (the calibrated drops for positive stock was 14 drops/ ml).

Volume of Negative stock required = 5-2.5 = 2.5 ml or 15 x 2.5 = 38 drops (the calibrated drops for negative stock was 15 drops/ ml).

Hence, 35 drops of the positive stock suspension is mixed with 38 drops of the negative stock suspension. Smear is made from this 3+ suspension and the actual AFB, pus cells and number of drops per ml are counted.

Similarly to prepare 5 ml of 2+ grade smear with 5 AFB/field from the positive stock suspension (40 AFB/ field),

N = (5AFB/40AFB)*5 = 1 ml of positive stock suspension is required. Hence, 14 drops of the positive stock suspension is mixed with 4 ml or 60 drops (15 x 4 = 60 drops) of the negative stock suspension.

Or else the 2+ suspension can also be prepared using the 3+ suspension. i.e., here AC=20, DC = 5, N = (5/20)*5 = 1.25 ml. Hence, 18 drops (14 x 1.25) of the 3+ positive suspension is mixed with 56 (15 x 3.75 = 56) drops of the negative stock suspension.

Similarly from this 2+ suspension, 1+ grade suspension and from 1+ grade suspension scanty grade suspensions can be prepared.

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 NALC) when the amount of leukocytes is relatively high and avoid dilution if the amount of leukocytes is low.

Preparation and Validation of Batches of Slides

a. Using diluted stock preparations, 50-100 slides per batch are prepared. 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. If some samples that are produced and tested are not of sufficient consistency (see Table*), the whole batch from which the sample was derived should be discarded.

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. It is recommend that laboratories prepare 50-100 slides so that sufficient slides are available 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 it is estimated 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.

Standard deviation: The standard deviation is computed from slide test results 1-6. The following formula is used to compute Standard deviation.

$$\sqrt{\frac{n\sum x^2-(\sum x)^2}{n(n-1)}} \qquad \textit{Where } x = \textit{average and } n = 6$$

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

Mean [M] minus 2 standard deviations [SD] (M-2SD) and Mean [M] plus 2 standard deviations [SD] (M+2SD). The following Table gives the acceptable values for each grade of smear for RNTCP for declaring the batch as ‘acceptable’ for Panel Testing.

Grade	M-2SD	M+2SD	Consistency
3+	≥11/ field		True (sufficient)
2+	≥1/ field	≤10/ field	
1+	≥10/ 100 fields	≤99/ 100 fields	
Scanty	≥1/ 100 fields	≤9/ 100 fields	
Negative		0 / 100 fields	

If the value of 'M-2SD' or 'M+2SD' is outside the values suggested above, 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.

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.

On-Site Evaluation Checklist for NRL Laboratory Personnel to IRL

I General Information

Intermediate Reference Laboratory:	
State:	
Number of Microbiologists:	
Number of Lab Technicians:	
Name and qualifications of current staff: (Separate sheet to be attached to indicating information for each of lab staff, if it is different from the previous report)	
Head of IRL :	
Date of Visit:	
Visiting NRL Supervisor:	

II Action required as per the previous visit:

III Current visit particulars

Sl. No	Item	Adequate / Acceptable *	Problems Identified
1	**Infrastructure:** Separate area for TB laboratory work Separate tables for specimen receipt/smear preparation/ microscopy	Y / N	
2	Power supply	Y / N	
3	Running water supply	Y / N	
4	Microbiologist: Training in RNTCP/ EQA	Y / N	
5	LT: Number and training in RNTCP/ EQA	……..Y / N	
6	**Standard Operating Procedure:** Display and follow smear preparation and staining procedure	Y / N	
7	Display and follow grading chart	Y / N	
8	EQA Protocol available and followed	Y / N	
9	**Adequate stock and supply of:** Slides	Y / N	
10	Lens Tissue	Y / N	
11	Filter paper	Y / N	
12	Spirit lamp or Bunsen burner	Y / N	
13	Immersion oil	Y / N	
14	Disinfectants	Y / N	
15	Smearing/staining equipment (staining racks, loops, sticks etc)	Y / N	
16	Slide boxes	Y / N	
17	EQA forms	Y / N	
18	**Staining reagents / equipment:** Carbol fuchsin	Y / N	**Within expiry date Y / N**
19	Methylene Blue	Y / N	
20	25% Sulphuric acid	Y / N	
21	Distilled water	Y / N	
22	Equipment for preparation of stains/ reagents such as balance (for weighing reagents), measuring cylinders etc	Y / N	
23	Equipment for preparation of panel testing slides	Y / N	
24	**Binocular Microscopes**	Y / N	
25	**Disposal of infected material:** Waste containers with lid	Y / N	
26	Waste disposal by Autoclave/disinfection/buried	Y / N	
27	General order/cleanliness	Y / N	
28	**Safety Practices**	Y / N	
29	**Training status:** Any change in staff since last supervisory visit.	Y / N	
30	Has each IRL supervisor undergone training/ refresher training in EQA within past two years	Y / N	

Sl. No	Item	Adequate / Acceptable *	Problems Identified
31	**Internal Quality Control:** Control smears are used for each new batch of stain	Y / N	
32	**External quality control:** All DTCs are visited at least once by IRL staff, as per their tour programme for the year	Y / N	
33	Preparation of panel testing slides: Number sufficient for all districts of the state	Y / N	
34	Validation of panel testing: Number and range of AFB and pus cells	Y / N	
35	Are all slides kept as required by the RNTCP EQA Programme? (Unstained Panel slides kept as per their batch number and grading after validation)	Y / N	

*** Standards for reagents given at annexure-H**

IV Onsite panel slides rechecking (attach separate sheets, if required)

a) Panel slide testing is conducted once a year at this level, coinciding with on-site evaluation. Rechecking is done for all discordant results of panel testing slides from NRL.

i) Evaluation of manufacture of panel slides at IRL (review the validation process also)

Slide. No.	Result of designated state level lab technician	Result of National level laboratory	Staining AFB and background	Remarks (including review of validation process)

ii) Results of panel testing at DTC acceptable Y / N

iii) If no give details:

ii) Panel testing results of IRL using manufactured panel slides from NRL for each laboratory technician and Microbiologists of IRL

To be entered by IRL LT		For use by National Reference Laboratory Technician		
Slide number	**Result**	**Expected result**	**Error type**	**Remarks**

To be entered by IRL LT		For use by National Reference Laboratory Technician		
Slide number	**Result**	**Expected result**	**Error type**	**Remarks**

Comments

b) Assessment of EQA responsibilities of IRL for district-level laboratories under them

EQA Activity of IRL	Number to be performed during the assessment period*	Number actually performed	Remarks
On-site evaluation			
Panel testing			

* Assessment period refers to the period from first day of the year till the current date

V Review IRL on-site and panel testing results of DTCs (refer to report and feedback form)

a) Have performance problems (based on criteria set by RNTCP) been identified through on-site evaluation and panel testing? Yes No

b) If yes, what corrective actions been recommended:

c) Has corrective action been adequately implemented (check feedback reports from DTC's)? Yes No

c) If no, explain:

On-site evaluation summary for IRL

(Copy of the below mentioned summary to be left with IRL Director)

Name of NRL	
Name of IRL:	
State:	
Date of Visit: (dd/mm/yyyy)	
Visiting NRL Supervisor:	
Action required as per the previous visit:	

VI Summary of current visit:

a) Operational problems (pending as well as new)

b) Technical problems (pending as well as new)

c) Overall remarks

d) Action Required

Signature of the visiting NRL team leader with date

Signature of IRL Director with date

On-site evaluation Quarterly report of NRL to CTD

NRL			
Quarterly Report			Quarter _____ Year _____
A) IRLs visited with dates of visit and names of NRL Supervisor(s)			
Sl No.	**Name of the IRL**	**Dates of visit**	**Name(s) of NRL Supervisor(s)**

B) - Report on panel testing done during on-site evaluation

(After rechecking of discordant slides)

Sl. No.	IRL-LT	HFP	HFN	LFP	LFN	QE	Total number of errors

C) - Details of corrective actions recommended and taken			
Sl. No.	**IRL**	**Recommended corrective actions / Corrective actions taken**	**Remarks**

D) Any other remarks.

Signature of the Director,
National Reference Laboratory.

EQA NETWORK IN SPUTUM SMEAR MICROSCOPY UNDER RNTCP

On-Site Evaluation Checklist for Evaluation of District Level Laboratory

I General Information

DTC:	
State:	
Number of STLS:	
Name and qualifications of current staff: (Separate sheet to be attached indicating information for each of STLS, if it is different from the previous report)	
DTO:	
Date of Visit:	
Visiting IRL Supervisor:	

II Action required as per the previous visit:

III Current visit particulars

Sl. No	Item	Adequate / Acceptable	Problems Identified
1	**Infrastructure:** Separate area for EQA Lab work Separate tables for re-staining and smear microscopy for RBRC	Y / N	
2	Power supply	Y / N	
3	Running water supply	Y / N	
4	STLS: Training in RNTCP/ EQA	Y / N	
5	LT: Number and training in RNTCP/ EQA		
6	**Standard Operating Procedure:** Follow smear preparation and staining procedure	Y / N	
7	Follow grading chart	Y / N	
8	Follow EQA Protocol	Y / N	
9	**Adequate stock and supply of:** Slides	Y / N	
10	Lens tissue	Y / N	
11	Filter paper	Y / N	
12	Spirit lamp or bunsen burner	Y / N	
13	Immersion oil	Y / N	
14	Disinfectants	Y / N	
15	Smearing/staining equipment (staining racks, loops, sticks etc)	Y / N	
16	Slide boxes	Y / N	
17	EQA forms	Y / N	
18	**Staining reagents / equipment:**		Within expiry date
19	1% Carbol fuchsin	Y / N	Y / N
20	0.1% Methylene Blue	Y / N	Y / N
21	25% Sulphuric acid	Y / N	Y / N
22	Distilled water	Y / N	Y / N
23	Equipment for preparation of stains / reagents such as balance (for weighing reagents) and measuring cylinder etc	Y / N	
24	**Binocular microscopes**	Y / N	
25	**Disposal of infected material:** Waste containers with lid	Y / N	
26	Waste disposal by Autoclave / disinfection / buried	Y / N	
27	General order/cleanliness	Y / N	
28	**Safety Practices**	Y / N	

Sl. No	Item	Adequate / Acceptable	Problems Identified
29	**Training status:** Any change in staff since last supervisory visit.	Y / N	
30	Has each STLS undergone training/ refresher training in EQA within past two years	Y / N	
31	**Internal Quality Control:** Are all STLS using positive and negative control slides for internal quality control of each new batch of stain as required by the RNTCP?	Y / N	
32	**External quality control:** All DMCs are visited at least once in a month by STLS during the current period of the year	Y / N	
33	Are all slides kept as required by the RNTCP EQA Programme?	Y / N	

IV Onsite Panel slides rechecking: (attach separate sheets, if required)

A) Panel slide testing is conducted once a year at this level, coinciding with on-site evaluation. Rechecking is done for all discordant results of panel testing slides only.

Staining technique results

Slide. No.	Staining AFB and background	Remarks

Panel testing results

To be entered by STLS		For use by IRL LT		
Slide number	**Result**	**Expected result**	**Error type**	**Remarks**

Comments

B) Assessment of EQA responsibilities of STLS.

EQA Activity of STLS	Number to be performed during the assessment period*	Number actually performed	Remarks
On-site evaluation			
Blinded Re-checking			

* Assessment period refers to the period from first day of the year till the current date

V Blinded Re-Checking Results (refer to reports)

a) What is the expected number of slides to be checked by all STLS during the month?……..

b) How many have been checked?..... Percentage checked ……%

c) Was the blinding procedure supervised by DTO?.....

d) Type and number of errors detected:……

……..

e) If errors are detected, explain recommended actions for each DMC:

f) Has corrective action been adequately implemented (check STLS reports)? Yes No

g) If no, explain:

On-site District Level Lab summary of IRL

(Copy of the below mentioned summary to be left with DTO)

Name of IRL	
Name of DTC:	
District:	
Date of Visit: (dd/mm/yyyy)	
Visiting IRL Supervisor:	
Action required as per the previous visit:	

VI Summary

a) Operational problems (Pending and new)

b) Technical problems (Pending and new)

c) Overall remarks

d) Action Required (Use extra sheets if required)

Signature of IRL team leader with date

Signature of DTO with date

On-site quarterly evaluation report of DTCs visited by IRL Team (to be given to concerned STO, IRL, and NRL)

IRL:			
Quarterly Report			Quarter _____ Year _____
A) DTCs visited with dates of visit and names of IRL Supervisor(s)			
Sl No.	**Name of the DTC**	**Dates of visit**	**Name(s) of IRL Supervisor(s)**

B) Report on panel testing done during on-site evaluation:

(After rechecking of discordant slides)

Sl. No.	STLS	HFP	HFN	LFP	LFN	QE	Total number of errors

C) Details of corrective actions recommended and taken			
Sl. No.	**STLS**	**Recommended corrective actions / Corrective actions taken**	**Remarks**

D) Any other remarks.

Signature of the Director, IRL.

On-Site Evaluation Checklist for STLS

I General Information

DMC:	
District:	
Number of Technicians:	
Qualifications of current staff: (Separate sheet to be attached to indicating information for each of Lab staff, if they are different from the previous visit)	
Supervisor/MO of DMC:	
Date of Visit:	
Name of visiting STLS:	

II Data on Slide volume for the last month:

This information is necessary to (i) select slides for Blinded Rechecking for the current month and as cumulative number for (ii) next annual SPR, (iii) next annual negative slides and (iv) annual total slides.

Sl. No.	Type of slide (Includes diagnosis and follow up slides)	Number
1	Positive slides	
2	Negative slides	
3	Total	

III Action required as per the previous visit:

IV Current visit particulars

Sl. No	Item	Adequate/ Acceptable	Problems Identified
1	Standard Operating Procedure (charts, manuals and modules)	Y / N	
2	Separate area for TB Lab work	Y / N	
3	Separate platform / tables for specimen receipt / smear preparation / microscopy	Y / N	
4	Power supply	Y / N	
5	Running water supply	Y / N	
6	Waste containers with lid	Y / N	
7	Waste disposal by Autoclave/burning/buried	Y / N	
8	**Adequate Stock and Supply of**: Specimen cups	Y / N	
9	Slides	Y / N	
10	Lens Tissue	Y / N	
11	Spirit lamp or Bunsen burner	Y / N	
12	Filter paper	Y / N	
13	Smearing / Staining Equipment (staining racks, sticks etc)	Y / N	
14	Slide boxes	Y / N	
15	Staining reagents:	Y / N	
15 (a)	1% Carbol fuchsin	Y / N	**Within expiry date Y / N**
15 (b)	25% Sulphuric acid	Y / N	**Within expiry date Y / N**
15 (c)	0.1% Methylene Blue	Y / N	**Within expiry date Y / N**
16	Immersion oil		
17	Label on sputum container	Y / N	
18	New slides used for AFB microscopy	Y / N	
19	Slides labeled with Lab Sl. No.	Y / N	
20	Number of specimens collected for diagnosis and for re-examination for diagnosis	Y / N	
21	Number of specimens collected for follow up examination	Y / N	
22	Smears air-dried prior to fixing	Y / N	
23	Staining procedure	Y / N	
24	Follow grading chart	Y / N	
25	Are positive results entered in Red ink	Y / N	
26	Control smears are used for each new batch of stains received at DMC	Y / N	
27	Binocular Microscopes	Y / N	
28	Maintenance of microscope	Y / N	
29	Laboratory Register	Y / N	

Sl. No	Item	Adequate/ Acceptable	Problems Identified
30	Write TB number of 'Follow up' patients in all cases	Y / N	
31	Write TB number and category of smear positive patients in the remarks column when this becomes available	Y / N	
32	Laboratory forms	Y / N	
33	Any change in lab staff since last supervisory visit.	Y / N	
34	Personnel	Y / N	
35	Training status	Y / N	
36	Has each staff member participated in refresher training within past two years	Y / N	
37	Safety Practices	Y / N	
38	General order / cleanliness	Y / N	
39	Timely reporting of results to clinicians	Y / N	
40	Does the TB Register contain all smear positive patients recorded in the TB Lab Register	Y / N	
41	Are the smear results for follow up patients in the TB Lab Register the same as the results recorded in the TB Register	Y / N	

42	Are all slides kept as required by the RNTCP EQA Programme?	Yes	No
43	Are slides collected for EQA, do the number in the slide box correlate with the number in the Lab Register	Yes	No

V Review of five positive and five negative slides from RNTCP TB Lab Register:

(Systematic sampling, separately for positive and negative slides)

a) Of the 5 Pos slides, number re-read as positive by STLS _____

b) Of the 5 Neg slides, number re-read as negative by STLS _____

Tick appropriate column or write letter as indicated below table

Sl. No.	Slide No.	AFB result / Grade by		Specimen Quality		Staining		Size		Thickness		Evenness	
		STLS	LT of DMC	≥10 WBC/ field	. < 10 WBC/ field	Good	Poor (U/O)	Good	Poor (B/S)	Good	Poor (K/N)	Good	Poor
		1		2		3		4		5		6	
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													

1: Write smear and grade
2: Tick appropriate column
3: Tick if good; write 'U' if under-decolourized, 'O' if over-decolourized
4: Tick if good; write 'B' if too big, 'S' if too small
5: Tick if good; write 'K' if too thick, 'N' if too thin
6: Tick appropriate column

*** Please carefully review all discordant slides with the LT**

Overall summary (please tick appropriate alternative):

Specimen quality: Needs improvement ❑ Yes ❑ No
Smear size: Needs improvement ❑ Yes ❑ No
Smear thickness: Needs improvement ❑ Yes ❑ No
Smear evenness: Needs improvement ❑ Yes ❑ No
Staining: Needs improvement ❑ Yes ❑ No

Name of STLS: _____ Signature of STLS: _____

Name of LT: _____ Signature of LT: _____

Name of MO-in-charge: _____ Signature of MO-in-charge: _____

Date_____

On-site evaluation summary of EQA of Smear Microscopy of DMC by DTO

(a copy of this summary to be submitted by DTO to MO of DMC for corrective actions)

DMC:	
Date of visit: (dd/mm/yyyy)	
Visiting STLS:	
Action required as per the previous visit:	

Summary

a) Operational problems (both pending and new)

b) Technical problems (both pending and new)

c) Overall remarks

d) Action Required

Name of STLS: _____ Signature of STLS: _____

Date_____

Remarks by DTO

Signature of DTO

Copy to CMO of the District

ANNEXURE B

RNTCP

Smear Results Sheet for Blinded Rechecking

Microscopy Centre: _____ District: _____

Name of TU_____ Month/Year: _____

Sl. No.	Lab No.	Result of LT of DMC, including grade for positive smears
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
15.		
16.		
17.		
18.		
19.		
20.		
21.		
22.		
23.		
24.		
25.		

Name of Lab. Technician: _____

Signature: _____ Date _____

ANNEXURE C

RNTCP EQA of Sputum Microscopy

Worksheet: Blinded Rechecking of DMC Slides

Microscopy Centre Code: _____ **TU_____**

District: _____ **Month and Year: _____**

Tick appropriate column or write letter as indicated below table

Sl. No.	Slide No.	AFB result / Grade by			Specimen Quality		Staining		Size		Thickness		Evenness	
		STLS	MC	Umpire	≥10 WBC/ field	< 10 WBC/ field	Good	Poor (U/O)	Good	Poor (B/S)	Good	Poor (K/N)	Good	Poor
		1			2		3		4		5		6	
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														
16														
17														
18														
19														
20														
Total														

1: MC result to be entered under supervision of DTO ***only after*** form completed by STLS
2: Tick appropriate column
3: Tick if good; write 'U' if under-decolourized, 'O' if over-decolourized
4: Tick if good; write 'B' if too big, 'S' if too small
5: Tick if good; write 'K' if too thick, 'N' if too thin
6: Tick appropriate column

Overall remarks:

Specimen quality: Needs improvement ❑ Yes ❑ No
Smear size: Needs improvement ❑ Yes ❑ No
Smear thickness: Needs improvement ❑ Yes ❑ No
Smear evenness: Needs improvement ❑ Yes ❑ No
Staining: Needs improvement ❑ Yes ❑ No

Remarks:

Date of examination: _____ Signature of first controller: _____

ANNEXURE D

RNTCP

Quality Assurance Report on Sputum Microscopy

Microscopy centre**:** _____ TU and District:_____

Month/Year :_____

Result of MC-LT	Result of controller *				
	Negative	1-9 AFB/ 100 fields	1+	2+	3+
Negative	Correct	LFN	**HFN**	**HFN**	**HFN**
1-9 AFB/ 100 fields	LFP	Correct	Correct	QE	QE
1+	**HFP**	Correct	Correct	Correct	QE
2+	**HFP**	QE	Correct	Correct	Correct
3+	**HFP**	QE	QE	Correct	Correct

* Enter the number of slides on each box

No. of False result		Slide No. / Error
False (-)ve		
False(+)ve		

Name and signature of STLS of concerned DMC:_ _____ _____

Reporting Date:_____ Signature of DTO_____

On-site evaluation Quarterly report of EQA from DTOs to IRL

District:	
Quarterly Report	Quarter _____ Year _____

Details of corrective actions recommended and taken			
Sl. No.	**DMC**	**Recommended corrective actions and corrective actions taken**	**Remarks**

D) Any other remarks.

_____

_____

_____

_____

_____

Signature of the DTO

Annexure E

District monthly report to IRL on random blinded rechecking

Sl. No.	DMC name	Annual slide volume*	Annual positive slides*	Slide positivity rate (SPR)*	Nos. of slides rechecked during the month@	HFP	HFN	LFP	LFN	QE	Total number of errors	Remarks
Total for the district												

* For the previous year.

@ Monthly sample size calculated from Annual sample size for 80% Sensitivity, 100% Specificity and 'd'=0 and Confidence limit = 95%.

Annexure F

On-site evaluation Quarterly report of EQA from DTOs to IRL

District:	
Quarterly Report	Quarter _____ Year _____

Details of corrective actions recommended and taken			
Sl. No.	**DMC**	**Recommended corrective actions and corrective actions taken**	**Remarks**

D) Any other remarks.

Signature of the DTO

Proposed changes to annexure F

Onsite evaluation & RBRC quarterly report of EQA from DTOs to IRL

District: **First controllers:**

Qarter: **Second controller:**

Year: **DTO:**

Sl.No.	Name of DMC	Number of smears examined in the quarter in DMC*			Number of smears rechecked in the quarter at DTC#			Number of errors made from DMC-LT					Major OSE corrective recommendations and implementation status
		Pos	1-9 AFB	Neg	Pos	1-9 AFB	Neg	HFP	HFN	LFP	LFN	QE	
Total													

First controller$ evaluation compared to Umpire**

Name of First controller	No. of Positive smears by first controller	No. of Scanty smears by first controller	No of negative slide by first controller	Number of errors made by 1st controller					Remarks: corrective actions, in case of errors by 1st controller
				HFP	HFN	LFP	LFN	QE	

* Based on the data from the lab register captured in monthly OSE forms**;** # **B**ased on annexure B and C;

$ STLS; **Based on the annexure C data

Annexure G

IRL annual report to CTD and NRL on random blinded rechecking

Sl. No	Name of the district	Total number of DMCs	INFORMATION ON SLIDES								INFORMATION ON DMCs			% of DMCs with HF errors	% of DMCs with HFP errors
			Annual slide Volume*	Annual positive slides*	No. of slides rechecked during year@	Number and Type of errors in slides Rechecked, cumulative in the district during the Year					Number of DMCs in the district with HF errors**				
						HFP	HFN	LFP	LFN	QE	HFP	HFN	BOTH HFP & HFN		
Total															

* For the previous year

**Each HFP or HFN error committing DMC is counted only once in the reporting year

@LQAS Annual sample size for 80% sensitivity,100%Specificity and d=0 confidence limit=95%

Annexure H

EQA Guidelines

1) Standards for Reagents
    a. Specifications:
        i. Basic fuchsin
            1. The chemical name: Pararosaniline hydrochloride
            2. The chemical structure: $C_{19}H_{18}N_3Cl$
            3. Molecular Wt: 323.8
            4. Colour: Metallic green
            5. Dye content: Should be available on the container. Approximately 85% - 88% (to calculate the required amount of Basic fuchsin, divide the actual amount by dye content. For example: Dye content = 85%, actual amount = 10 gms, required amount = 10/0.85 = 11.76 gms.)
        ii. Carbolic acid:
            1. The chemical name: Phenol
            2. The chemical structure: C_6H_5OH
            3. Molecular Wt: 94.11
            4. Melting point: $40^oC\pm2$
            5. Purity: 99.5%
            6. Please note: The critical concentration of Phenol in Carbol fuchsin is 5%.
            7. Phenol is highly corrosive, handle with extreme care.
        iii. Methylated spirit
            1. Chemical name: Ethanol denatured + 5% Isopropyl alcohol + 5% Methanol
            2. Molecular structure: C_2H_5OH
            3. Molecular wt: 46.07
            4. Purity: 90%
        iv. Sulphuric acid:
            1. Chemical structure: H_2SO_4
            2. Molecular wt: 98.08
            3. Purity: 95-97%
            4. Colour: Clear
        v. Methylene blue:
            1. The chemical name: Methylthionine chloride
            2. The chemical structure: $C_{16}H_{18}ClN_3S$.
            3. Molecular Wt: 319.9
            4. Dye content: Should be available on the container. Approximately 82% (to calculate the required amount of Methyl blue, divide the actual amount by dye content. For example: Dye content = 82%, actual amount = 1 gms, required amount = 1/0.82 = 1.22 gms.)
    b. Immersion oil:
        i. Immersion oil supplied by the manufacturer of microscope with refractive index closer to that of Glass or 1.515
        ii. Liquid paraffin (heavy), refractive index of 1.48, a colourless, odourless, transparent, free from fluorescence in day light with relative density of 0.827 to 0.890, viscosity of 110 to 230 mPa s., specific gravity of 0.76-0.78 at 15.5^oC.

2) Shelf life of prepared reagents: Carbol fuchsin, sulphuric acid, methylene blue reagents may be kept for a maximum period of 4 months.

3) Identification: All reagents should have a label with name of the reagent, name of the TU, name of MC, the date of preparation and the expiry date. The containers of Carbol fuchsin, Sulphuric acid, Methylene blue reagents should in addition have the name of the person preparing the reagent. Freshly prepared reagents should not be mixed with old stock.
4) Equipment:
    a. Slides:
        i. Size: 76 mm x 26 mm,
        ii. Thickness: 1.3 mm
        iii. Edges: Polished
        iv. Sealed in a moisture absorbing dessicant pack
    b. Balance:
        i. Type: Electronic or Analytical balance
            1. Electronic balance:
                a. General purpose table top laboratory balance, 220-230 V, stainless steel platform, keypad auto calibration function, auto off, prolonged battery life, overload and under load, low battery LCD indicator.
                b. Range: Wide range, 0.01 – 120 gms, (two digit decimal)
                c. Resolution: 0.01 gm
            2. Analytical balance:
                a. Enclosed in a glass box with shutters, dimensions of the box in cms: 46 x 34 x 20
                b. Oscillator type of balance, with levelling screws, two aluminium pans, plumb line for adjusting horizontal level
                c. Weighing capacity: 1 mg to 200 gms, with fractional weight and regular weight in boxes including rider and forceps to handle weights.
    c. Binocular microscopes:
        i. Specifications: As per Expert Committee recommendations.

Annexure J

TECHNICAL SPECIFICATIONS OF BINOCULAR BRIGHT FIELD MICROSCOPES
(Revised National Tuberculosis Control Programme)

A. PREAMBLE

Binocular Microscopes are required for detecting acid fast bacilli in sputum smear and other materials for use in Tuberculosis Control Programme laboratories, including those at Peripheral Health Centres.

The usage requires long hours of viewing through the microscopes.

B. SPECIFICATIONS

1.	Body	Binocular, sturdy, stable base body with focus adjustment controls in a position comfortable for prolonged use. The body should be powder coated.
2.	Eye piece	Paired, high quality, (image of the object as seen through the binocular eyepiece should be well defined centrally in least 2/3 field of view), achromatic, widefield, 10x without in built pointer. The eyepiece should be aplanatic and have a minimum field number of 18. Diopter adjustment must be present on one/both eye pieces or on the eye piece tube.
3.	Objectives	Three objectives: 10x, 40x, 100x, 10x and 40x objectives should have numerical apertures of 0.25 and 0.65 respectively and should be of spring loaded type or otherwise. 100x should have numerical aperture of 1.25 and should be of oil immersion and spring loaded type. Suitable prominent marking should be provided on 100x for easy identification. Unbreakable containers to be provided for storing the objectives. All objectives should be widefield, achromatic and parfocal. Marking for the Objectives Each objective should be engraved with the following information:- a) Name/insignia of the manufacturer. b) Magnification and numerical aperture, for example, 10x/0.25. c) 100x objective should be engraved with the word 'Oil' In changing from one objective to another or reintroducing the same objective by rotation of the nosepiece, the object at the center of the field should not appear displaced by more than 0.02mm in the object plane in any direction.
4.	Nose piece	Revolving nose piece to accommodate a minimum of three objectives with click stops. It should be provided with ribbed grip for easy rotation mounted on a precision ball bearing mechanism for smooth and accurate alignment. Extra ports if any should be fitted with dust proof metallic/ebonite caps.
5.	Stage	Uniformly horizontal, mechanical stage having dimensions of length 140mm (+ 20mm) & breadth 140mm (+ 20mm) with fine vernier graduations (minimum reading accuracy of 0.1mm). The stage should be provided with spring loaded slide holder for exact positioning of

		specimen/slide. It should be designed with convenient sub-stage vertical coaxial adjustment for slide manipulation. The stage should have ball bearing arrangement to allow smooth travel in transverse directions i.e. 80mm (+ 5mm) and front to back direction, 50mm (+ 5mm).
6.	Sub-stage condenser	Abbe-type condenser, numerical aperature (N.A) 1.25, focusable with rack and pinion arrangement incorporating an aspherical lens and an iris-diaphram. The condenser should have a filter holder and removable/swing in/out blue filter (suitable for bright field Microscopy)
7.	Sub-stage illuminator	1. The system should have a built-in variable light source (Illuminator). This light source should have a 20W, 6V Halogen lamp. The circuitry for the light source should include a constant voltage supply. The system should be provided with a step down transformer and an on/off switch and intensity control. The lamp should be provided with a lamp socket which has the facility for easy replacement of the bulb. The housing of the light source should be such that it will prevent dispersion of light and heating up of the body of the microscope. 2. Power supply - voltage:220V, 50 Hz AC - should have one on-off power switch, 3 core power cord with a 3 point male plug. 3. The system should have an inbuilt protective/safety device to withstand fluctuations of voltage from 140V to 280V. 4. A plano-concave mirror in fork mounting should be supplied which would be attachable to the base of field use. (where power is not available.) 5. The fuse for the halogen lamp should be easily accessible to the operator. 6. The Illuminator should have a built-in field diaphragm for Kohler illumination.
8.	Eye piece tubes	Binocular eye piece tubes, inclined at 45 degrees, rotatable through an angle of 360 degrees, having inter-pupillary distance range of 54-74 mm or wider, covering the above mentioned range.
9.	Focusing knob	Co-axial coarse and fine focusing knobs capable of smooth fine focusing movement over the full range of coarse travel. The fine focusing movement should have sensitivity of two microns or less (finer) over the entire coarse focusing range. The focusing knob should be on both sides. A focusing stop safety arrangement should be provided.
10.	General	i) All optical parts including objectives, eye pieces and prisms should have anti-reflective coating which also gives anti-fungal property. ii) All metallic parts should be corrosion-proof, acid-proof and stain-proof. iii) All parts of the microscope (including removable parts) should have insignia of the manufacturer engraved on it. iv) The supplier will supply the complete assembled microscope in a wooden box along with dust free cover. The box carrying the microscope should be made of well-seasoned wood or teak ply or board. The box should be suitably padded from inside of eliminate the risk of shock during transportation. It should be complete with lock and key arrangement, a suitable locking screw for securing the

		microscope and a cross-piece to retain it in position during transit. The box should be of an appropriate design with a carrying handle at the top and appropriate internal receptacles for holding the objectives, eyepieces and accessories. It should contain a bag of activated silica gel to keep the interior moisture-free. v) Each assembled microscope should be accompanied by an authorized list of accessories and spare parts. vi) Technical brochure (catalogue) and working manual should be provided with each microscope. vii) A bottle of at least 25ml immersion oil, a roll of lens tissue paper and lens cleaning solution (100ml) should be provided with each microscope. viii) One piece of anti static cleaning brush should be provided with each microscope for cleaning purpose. ix) Each microscope should be supplied with Blue filter. The blue filter should be packed in the box and not fixed on the microscopes.
11.	Spare parts	Each microscope should be supplied with spare parts as under: (as mentioned in Schedule of Requirement) i) 100x oil immersion objective (as per the specifications given under B3)-One ii) Halogen bulb, (6 volts, 20w)-6 Nos. iii) Fuses-6 Nos.
12.	Warranty	Performance warranty of three years from the date of supply. For any malfunction, the supplier shall replace the parts or repair the same at the user site free of cost within 15 days of the receipt of the complaints. During warranty period all services/replacement ensuring smooth functioning of the Microscopes must be done free of cost by the supplier.
13.	Requirement of service centre for after sales services	The supplier should have adequate after sale service facilities covering all region of the country. They should have the infrastructure and trained manpower to attend to any complaints within 15 days of receipt of the complaint.
14.	Testing & calibration	i) The successful vendor should supply a type test-certificate of the relevant optical & mechanical tests from a recognized competent authority at the time of supply. ii) The manufacturer/supplier shall provide duly calibrated (by accredited authority) measure instruments and demonstrate specifications for the purpose of inspection.

Annexure K

Investigation of Errors

Sl. No.	Pattern of errors	Possible causes	Suggested Investigation Steps
1	HFP and HFN	Unusable microscope	Examine a 3+ using that microscope
		Staining problems, poor stains, insufficient staining time or heating	Check stains and staining procedure
		Technician cannot recognize AFB	Test with clear-cut positive & negative slides and good microscope
		Gross neglect, overworked, lack motivation	Exclude other causes
2	HFP with or without LFP	Administrative error	Compare lab-register and verify correct slide number and result? Exclude causes of more frequent HFP, such as low concentration of sulphuric acid, unusable microscope, untrained or inexperienced LTs.
		Poor registration routine	Check accuracy of lab-register and other record keeping
		Staining problems/Fading	Check stains and staining procedure, consider re-staining for rechecking. Assess concentration of Phenol, Basic Fuchsin and Methylene blue.
		Technician unclear on AFB appearance	Look for inconsistent results of suspects (regularly single pos / low positive) in lab register
3	Many LFP, with or without occasional HFP	Problem with controllers Technician unclear on AFB appearance Contaminated stain/ reagents	Evaluate controllers Recheck sample of LFP from laboratory register Test stain with known negative smears, check the distilled water used for stain preparation
4	HFN with or without LFN	Administrative error	Compare lab-register with QC-listing: correct slide number & result?
		Very thick smears and/or poor light	Evaluate quality of smear preparation, check microscope
		Gross neglect	Exclude other causes
		Staining problems	Check stains and staining procedure, consider re-staining for rechecking. Assess concentration of Phenol, Basic Fuchsin and Methylene blue.
		Poor smearing-technique	Test stain with known negative smears
		Problems with microscope	Check microscope with positive slide
		Careless microscopy	Exclude other causes
5	Very high proportion LFN.	Reading error	As above
		Concentrated Methylene blue	
9	Many QE (too low grading)	Poor staining	
		Problems with microscope	

* Refer RNTCP LT Module, Manual and STLS Module for causes of False Positive and False Negative results.

Annexure L

Possible reasons and suggested corrective actions for DMCs with unacceptable ANSV and SPR

Sl. No	Finding	Possible reasons	Suggested corrective actions
1	Low ANSV (<500)	Inadequate referral of suspects,	Educate Medical Officers and Health Workers on chest symptoms
		Non-involvement of private sector	Involve private sector, without increasing the number of DMCs in the area
		Many DMCs in the area	Reassess the criteria for selection of DMCs. Generally health facilities with <60 daily new adult OPD attendance should not be involved as DMCs
2	Low SPR (<5%)	Improper or over selection of TB suspects	Educate Medical Officers and Health Workers on chest symptoms
		More of false negative smear results	Action as required for frequent HFN and LFN Re-stain and test with known positive slides
3	Low ANSV and SPR	As above	Evaluate as above and if required consider closing the concerned DMC
4	High ANSV (>5000)	High OPD attendance workload	Train more than one LT for AFB smear microscopy
5	High SPR (>15%)	Selective referral / delayed identification of TB suspects	Educate Medical Officers and Health Workers on chest symptoms
		More of false positive smear results	Action as required for frequent HFP and LFP / test with known negative slides

Annexure M

Tuberculosis Laboratory Monthly Abstract
(Record Numbers)

Month Year 200.	TB suspects examined for diagnosis	TB suspects found positive	TB suspects undergoing repeat sputum examination	TB suspects found positive on repeat examination	Follow-up patients examined	Patients positive in follow up	Total slides examined	Total positive slides	Total negative slides	Signature of LT and STLS
Jan										
Feb										
Mar										
Apr										
May										
Jun										
Jul										
Aug										
Sep										
Oct										
Nov										
Dec										
TOTAL										

Signature of the M.O

Glossary of terms

AFB Acid-fast bacilli

Blinded Rechecking Sending smears from the peripheral laboratory to a DTC laboratory for rereading. Rechecking must always be blinded, whereby the controller does not know the results from the peripheral laboratory.

Controller Term used to describe the Senior TB Laboratory Supervisor or any technician responsible for rechecking slides.

CTD Central TB Division

Designated Microscopy Centre (DMC) A laboratory located at a primary health centre or district hospital, designated by RNTCP as a facility which will provided smear microscopy services to an approximate population of 1,00,000.

District The administrative level at which the RNTCP is implemented. In an urban setting, this may be a Municipal Corporation.

District TB Centre (DTC) Nodal centre for TB control activities in the respective district

DTO District TB Officer

DOTS Directly Observed Treatment, Short course chemotherapy. The internationally recommended strategy for TB control. The strategy includes (1) government commitment to TB control activities, (2) case detection by sputum smear microscopy, (3) directly 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.

External Quality Assessment (EQA) A process that allows participant laboratories to assess their capabilities by comparing their results with those in other laboratories in the network (IRLs and NRLs) through on-site evaluation of the laboratory, panel testing and blinded rechecking.

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

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

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

Intermediate Reference Laboratory (IRL) Intermediate level laboratory existing usually in the State TB Training and Demonstration Centre (STDC). The IRL may exist as part of the state public health laboratory if no STDC existing in the State.

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

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

LQAS Lot Quality Assurance Sampling

LT Laboratory Technician

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.

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.

National Reference Laboratory (NRL) The NRLs **s**erve as the national level reference laboratories for the tuberculosis programme. They play an essential role in the RNTCP for the organization and maintenance of the network of laboratories, development of guidelines for standardizing smear microscopy, quality assurance, and overseeing training. In 2004, there are three NRLs identified for India, namely National Tuberculosis Institute, Bangalore, Tuberculosis Research Centre, Chennai and LRS Institute, New Delhi. However, every NRL has to obtain accreditation by the supra-national laboratory.

OSE On-site Evaluation

Panel Testing Sending stained and/or unstained smears from the NRLs to the IRLs and IRLs to DTCs / DMCs to check proficiency in reading and reporting.

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 batches of staining solutions.

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 continuous monitoring, identifying defects, followed by remedial action including retraining when needed, to prevent recurrence of problems. QI depends on effective on-site evaluation visits.

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

RBRC Random Blinded Re-Checking

Revised National Tuberculosis Control Programme (RNTCP) A national programme responsible for activities directed at controlling tuberculosis through integrated efforts with the general health services for implementing the DOTS strategy.

Scanty (Low Positive) Term used in this document to describe 1-9 acid-fast bacilli per 100 fields, which is the RNTCP standard for quantification. These results are reported to the physician as exact number of AFB seen.

Slide positivity rate (SPR) Proportion of positive slides among all those examined (diagnosis and follow-up) in a DMC over a defined period of time, usually one year.

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

STDC State TB Training and Demonstration Centre

STO State TB Officer

STLS Senior Tuberculosis Laboratory Supervisor

Ziehl-Neelsen Stain (ZN) Acid-fast staining method using carbol fuchsin and methylene blue.