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EXTERNAL QUALITY ASSESSMENT SYSTEM FOR SPUTUM SMEAR MICROSCOPY IN INDIA : OPERATIONAL AND TECHNICAL IMPLEMENTATION ASPECTS

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SUMMARY

The credibility, success, and sustainability of the Revised National Tuberculosis Control Programme (RNTCP) depend on the strength of the laboratory network. Sputum Smear Microscopy has been the corner stone of Tuberculosis (TB) diagnosis and disease monitoring in RNTCP. To achieve and maintain the national objectives of 85% treatment cure rate among new Pulmonary Sputum Positive TB cases, and to achieve and maintain detection of at least 70% of such cases, quality assurance of Sputum Smear Microscopy is most immediate need. Quality Assurance (QA) system enhances the performance of the laboratory by validating methods with Internal Quality Control (IQC) and External Quality Assessment schemes (EQA). IQC & EQA identifies inappropriate procedures, out-of-date reagents, uncontrolled instrumentation, and /or training needs of incompetent or untrained staff. EQA improves sputum smear microscopy performance by retrospective analysis of the laboratory's testing procedure. by comparison with other testing laboratories and a reference laboratory.

The main focus of revised EQA programme, in India is given to: (a) the On-site evaluation (OSE) of laboratories in a periodic fashion, (b) monthly Random blinded Rechecking (RBRC) of the routine Designated Microscopy Centre (DMC) slides using statistically valid sampling procedure- Lot Quality Assurance Sampling (LQAS)- for rechecking programme, (c) proficiency testing of supervisors with panel testing, and (d) strengthening the state level laboratories (labs) for evaluating the peripheral labs. The revised EQA is being implemented in a phased manner in the country. EQA-teams (one microbiologist and two senior lab technicians) of majority of state level labs have been trained. Specific operational and technical problems are continuously identified and addressed with corrective measures. An efficient EQA system would install confidence in RNTCP diagnostic algorithm by identifying and correcting the systematic operational and technical errors in the laboratories.

Abbreviations & Keywords: **AFB**-Acid Fast Bacilli; **DOTS**- Directly Observed Treatment, short course; **DMC**-Designated Microscopy center; **DTC**- District TB center; **EQA**-External Quality Assessment; **IQC**-Internal Quality Control; **IRL**-Intermediate Reference Laboratory; **LT**-Laboratory Technician; **LQAS**-Lot Quality Assurance sampling; **NRL**-National Reference Laboratory; **OSE**- Onsite Evaluation; **QA**-Quality Assurance**; QI**-Quality Improvement; **RBRC**-Random Blinded Rechecking of Routine slides; **RNTCP**-Revised National Tuberculosis Control Programme; **SOP**-

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Standard Operating Procedure; **STDC**-State TB Training and Demonstration center; **STLS**-Senior TB Laboratory Supervisor; **TU**- Tuberculosis unit; **ZN**-Zieihl-Neelsen method;

INTRODUCTION

Tuberculosis is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* and spreads through air. TB is diagnosed by the examination of three sputum smears ("Spot" "Morning" "Spot") under microscopy after staining by Ziehl Neelsen (ZN) method [1]. Every year 1.8 million new TB cases occur in the country of which 0.8 million are infectious. Every day, about 5000 people develop TB disease while over 1000 die of TB.[2]. Unless properly treated, infectious pulmonary TB patients can infect 10–15 persons in a year.[2]. Poorly treated patients can develop drug resistant and potentially incurable forms of TB.

RNTCP for treatment of TB is one of the most significant public health programmes in India[3]. RNTCP, based on the internationally recommended DOTS strategy [4], began in full-fledged manner in year 1998 and expanded to cover the country in a phased manner - Approximately 1030 million (93%) people were covered under RNTCP by second quarter 2005. DOTS strategy, which is adopted from the research conducted in prominent institutions in India, has five components: (1) Political and administrative commitment (2) Good quality diagnosis, through sputum Microscopy (3) Uninterrupted supply of good quality drugs (4) DOT (5) Systematic monitoring and accountability.

1. IMPORTANCE OF SPUTUM SMEAR MICROSCOPY AND QUALITY ASSURANCE

Sputum smear microscopy forms the most important technique for implementing two components in the RNTCP-DOTS strategy mentioned above, for initial TB diagnosis (second component 2 of DOTS); and follow-up of the treatment progression to undertake systematic monitoring of the programme (fifth component of DOTS). To maintain the national objectives of 85% treatment cure rate among new pulmonary sputum positive TB cases, and to detect and maintain at least 70% of such cases, quality assurance of sputum smear microscopy is the most immediate need. More than 10,000 peripheral labs / DMC have been established under the RNTCP for diagnosis of TB by smear microscopy for AFB.[2,5] Rapid expansion of RNTCP in treating the pulmonary TB cases needs to be sustained through good quality assurance measures so that in the race for expansion the quality is not compromised.[2]. Keeping this in view, Indian RNTCP has adopted recent international guidelines[6] (brought by association of public health laboratories in year 2002) into national EQA document to revise the quality control measures.

Consequences of False Results in TB diagnosis

Laboratory false Positive smear results lead to patient receiving unnecessary treatment with toxic drugs and in addition puts precious resources of the programme to drain, increasing the health care costs. False negative and false positive results lead to lose of confidence in RNTCP by Public and treating physicians. Accuracy or correctness of smear microscopy results needs an efficient Quality Assurance system, to identify operational and technical problems for false results. that are potential sources of error resulting in false results, and this ensures that appropriate corrective actions are initiated such that the error

does not occur in the future.

2. QUALITY ASSURANCE SYSTEM

2.1 Components of Quality assurance system

A Quality Assurance (QA) system for AFB smear microscopy includes the following three interdependent components: Quality Control (QC), External Quality Assessment (EQA) and Quality Improvement (QI)(Figure 1) [6, 7]

Quality control (QC) (or internal quality control measures (IQC)) is a systematic internal monitoring of infrastructure, working practices, technical procedures, equipment and materials, including quality of stains to maintain the smear microscopy technique to be accurate, reliable and reproducible ([6,7]. Infrastructure includes the appropriate lab space and equipment, adequately trained staff to handle the workload, maintenance of supplies, and bio-safety & infectious waste management procedures. QC is the responsibility of all laboratory workers. For example the LT should include the quality control smears for testing the quality of new reagents prepared supplied to him to check the reliability on a batch-by-batch basis. Table I details the QC measures for sputum collection, smear preparation, staining and reporting of results.

One of the most important IQC measure for the ZN staining reagents is the use & subsequent validation of unstained control test smears for each batch of regents prepared: a control test set made of two smears: a high positive (3+ grade: >10AFB/oil immersion field), and a negative (0 AFB/100 oil immersion fields).[5].

EQA involves systematic monitoring of the performance of laboratories and therefore in an unbiased fashion. Three functional components of EQA are: OSE, Panel testing and RBRC. EQA includes On-site evaluation of the laboratory is performed to review QC and allows participant laboratories to assess their capabilities. EQA also facilitates by comparison of the results with those obtained in other laboratories in the network (intermediate and national reference laboratoriesy) by employing methods such as Panel Testing and Blinded Rechecking of routine smears [6,7]

Quality improvement is a continuous process, which overlaps with the findings of QC and EQA to include the appropriate corrective actions taken at right time to avoid recurrence of systematic error. 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 visits.[6] .

2.2 Quality assurance Laboratory Network

RNTCP has developed national guidelines for India with specific hierarchical functions integrated within the RNTCP structure for EQA of sputum smear microscopy.[5]. RNTCP relies on sputum smear microscopy laboratory for diagnosis, categorization of patients and assessment of treatment progress. EQA implementation requires establishment of a well functioning laboratory network. The network consists of National level laboratories (called national reference laboratory, NRLs), state wise intermediate reference laboratories (called intermediate reference laboratory/ (located at State TB Demonstration and training Centers-(STDCs) are redesigned as IRL for this purpose), peripheral laboratoriess (Designated Microscopy Centers-DMCs). DMCs are monitored by Tuberculosis units (TU) and District TB Centers (DTCs). DMCs , that provides the population population with easy access to high quality smear microscopy services and are given the highest priority for RNTCP.

National wise EQA structure, organization and the responsibilities are summarized in table 2. Each Designated Microscopy Center caters to

an approximate population of 1,00,000. A TU caters ideally to the needs of 5 lakh population, and District Tuberculosis Center caters to the needs of 20 lakh population[2]. The network of peripheral laboratories (DMCs/TUs) is supported by larger regional intermediate reference laboratories (State TB training and demonstration centers) and National TB Reference Laboratories. The designations of the personnel involved at various levels is: DMC-Laboratory Technicain (LT) (at DMC); Senior TB Laboratory Supervisor (STLS) (at TU/DTC); DTO (at DTC); STO/STDC director, microbiologists and LTs of STDC (at STDC); national EQA supervisory team-Microbiologists, and Sr LTs and LTs at NRLs; and a national committee of experts (at national Central level)[5]

3. FUNCTIONAL COMPONENT OF EQA

Three functional components used for external quality assessment are OSE, RBRC and Panel testing (Table 3).

3.1. EQA On-site Evaluation

Supervision for quality assessment is conducted with prior intimation to the laboratories. A thorough Onsite evaluation is carried out with the help of a checklist (table 4), broad aspects listed in table 3 and suggestive corrective actions are recommended to the director/administrators of the laboratories.[6]. A comprehensive report to the higher level authorities is based on the feedback received regarding the implementation of the suggestive actions from the lab where OSE was conducted. OSE have demonstrated that some laboratories may not even be aware of their inability to adequately conduct testing. Alternately, the directors and/or administrators of a small number of facilities are aware of their poor performance, but attempt to continue to operate, due to lack of knowledge about the corrective actions.

The quality indicators for conducting OSE of STDC (intermediate reference laboratory) covering operational and technical problems are listed out in table 5.

3.2 Random Blinded Rechecking

RBRC of routine DMC slides, a simple and operationally more viable statistical sampling method called LQA sampling methodology has been adopted.[5] The operation feasibility of this approach under field conditions was established in pilot studies[6,7] conducted in Gondia and Bhandara districts of Maharashtra in September 2004, and at Thiruvallur district of Tamil Nadu in December 2004. A similar type of study conducted in Mexico also confirmed that RBRC provides more accurate estimates of AFB microscopy results, resulting in improved diagnosis and monitoring of treatment response[8]. Adopting the international guidelines for smear microscopy and LQAS parameters such as sensitivity of 80%, specificity of 100%, acceptance number 0, and confidence interval of 95%, Indian EQA-authorities developed a modified LQAS annual sample size table for RBRC (table 6).

STLS is directed by DTO to pick up a sample of slides depending on the total number of negative slides and slide positivity rate in the DMC in a year. These slides are deposited on a monthly basis at DTC where DTO takes responsibility of blinding and conducting the rechecking by the “controllers/umpire”. Table 7 forms the basis for validating the smear results. Results are reported on a monthly basis (in electronic mail/hard copy) to higher level labs and a feedback given to the peripheral labs about their performance profiles. High False results in RBRC forms a strong basis for DTO to visit and initiate corrective actions wherever they occur. The more frequent causes for errors in RBRC and suggestive corrective measures are listed in table 8.

Key features of the revised EQA guidelines are listed in the table 9. Prior to the new

guidelines, EQA 2001 guidelines [11] were implemented in the country in which, the random blinded cross checking (RBCC) of patients slides at TU level by examination of 20 slides every month; all positive and 10% of negative slides were cross checked in an unblinded fashion during the monthly supervisory visits[9]. This resulted in large workloads for the supervisors making it non-feasible under the operating conditions.

3.3 Panel testing :

Panel testing or Proficiency testing is conducted during the OSE visit i.e., under supervision. Panel testing is conducted for the lab personnel who are not doing routine sputum smear microscopy activities but are supervising the DMC LTs. The microbiologist and LTs of IRL and the STLS of the TU are the personnel who require panel testing to assess their proficiency (Table 3).

4. SPECIFIC OPERATIONAL PROBLEMS ENCOUNTERED IN EQA IMPLEMENTATION IN INDIA

- TB laboratory Supervisors in certain states are the permanent staff of the state government health services and thus are liable for the frequent transfers from the place of work, and health exigencies that occur from time to time. For example Malaria, cholera being seasonal (mainly monsoon) in certain endemic zones, entire TB supervisory staff is transferred for the malaria control purposes for a period of three months thus compromising the TB control & EQA activities. National immunization days for Polio eradication is another such constraint.
- Over population of certain states of the country does not allow sufficient decentralization of services as per RNTCP structure for District, sub-district and designated microscopy facility levels, bringing in a lot of implementation aspects regarding the maintenance of quality. The transition period between changes in the administrating agencies lead to a set of operational problems such as the total organization, procurement system for instruments, Annual Maintenance Contract of microscopes, staffing patterns, differences in conducting the rechecking programmes etc.
- Certain Union territories have peculiar geographic administrative enclave spread across more than 2-3 states making it difficult to supervise and implement RBRC as is envisaged in the guidelines.
- The state level laboratories are performing Clinical activities and primary patient care that compromises the supervisory authority vested in the EQA guidelines.
- At present, in many states STDC attached DMC laboratory is de facto State EQA lab leading to merging of supervisory structure and functions.
- Capacity building for laboratory infrastructure is urgently needed to manufacture quality panel smears for proficiency testing.
- The State TB Officer, who is overall administrative in-command of RNTCP, is also holding the additional responsibilities of Head of state level labs (Director, STDC) and district level labs (DTO) which directly compromises his supervisory role as per the EQA guidelines.
- Non-availability of qualified microbiologist in EQA team and at State level labs to oversee the laboratory activities.
- High false results in more than one instance are due to lacunae in the system. Priorities to high false results in RBRC need to be given by DTOs.
- Implementation of corrective actions and submission of action taken reports at various

levels is not adhered. The main role of EQA is not only in identifying errors but also in documenting them and taking corrective measures in a timely manner.

- The recent guidelines on infectious waste management of laboratories need to be implemented.

5. EQA REPORTING SYSTEM

The periodic reporting system adopted for EQA, at various levels of hierarchical structure, is summarized in the fig 2. OSE forms and reporting details are listed as annexure in the revised EQA document. The Panel testing results forms the part of OSE reports since the proficiency testing is carried out during the on-site evaluation visits. Monthly RBRC results from each district are sent in electronic mail format to Central TB Division and also to State TB training and Demonstration Centers. Rechecking results of routine DMC smears are also reported in the quarterly reports on programme management and logistics submitted by TU level (unblinded cross checking results) and District level (RBRC) structure to Central TB division. At present, the EQA on-site visits are not yet integrated with programme management report. In addition to these reporting structure, regular feed-back on actions suggested by the higher level labs for improvement of performance of the peripheral labs are sent in a timely fashion.

7. CONCLUDING REMARKS

QA of Smear microscopy is an essential component of TB control programme. External Quality Assurance conducted within the framework of RNTCP structure will avoid false positive and false negatives and helps in maintaining the accuracy of 'detection' and 'cure' rates of TB cases. Central TB division as per the revised international guidelines developed in India specific guidelines for EQA. The emphasis is given for OSE of the laboratories and blinded rechecking of routine slides of designated microscopy centers. Proficiency of the STLS is entrusted with the State TB training and Demonstration Centers. The programme is under implementation stage in a phased manner in the country. Operational and Technical problems of implementation are continuously analyzed and corrective measures are implemented at various levels. An effective EQA system paves way for the correctness of drug-resistance surveys and appropriate treatment of DOTS 'failure' patients under the extended DOT and DOTS-plus programmes.

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Table 1: **Internal Quality Control Measures**

Quality of….	**Measure**
Sputum Specimen	Muco-purulent; minimum 10-20 Pus cells/field; labels on cups/ slides/forms; time lapse between collection & processing of sputum
Smear Preparation	New slides; gentle heat fixing, labeled with lab number; Size; Thickness; Uniformity; Back ground

Quality of….	Measure
Stains	Potency/purity; label on bottle with name of reagent with date of preparation; QC of prepared Reagents (positive & negative controls
Staining	Over decolorized/under decolorized; over counter stained/ under counter stained; drying; leveled sink; As per RNTCP-SOP
Microscopy & smear examination	Microscope: Eye piece/x100 Objective/light source/maintenance; Immersion oil; Identification of AFB, results and grading; removing the immersion oil/preservation & storage/ serial order as per lab register.
Bio-safety & Disinfections	Safety of lab technician in sputum collection and processing; Disposal of sputum, sputum cups & caps; inoculation loops or swab-sticks; used sputum smear slides; Work-bench; 5% Phenol solution(do not use pre-prepared hypochlorite or bleaching powder)
Reporting of AFB Results	Documentation of results; Prompt and correct reporting as per RNTCP guidelines without any clerical errors

Table2: Structure of EQA and responsibilities (adopted from ref 2)

Structural Level	Responsibilities
National Reference Laboratories (NRLs): 1. National Tuberculosis Institute, Bangalore 2. Tuberculosis Research Centre, Chennai 3. LRS Institute of TB and lung diseases, New Delhi	o National Policy- EQA-guidelines development (methods, manual, QA protocols); o Training of all State level EQA personnel; o QA planning and implementation; o Capacity building of intermediate reference labs with regard to man-power, equipment and procurement; o Supervisory visits (OSE) to STDC and suggesting corrective action; o Proficiency testing of all State level EQA personnel; o Monitoring and assessing the performance of the EQA system in the country bringing in changes as and when needed
Intermediate reference laboratories (IRL) : State level laboratories located at STDCs of each state	o QA implementation in the State o Supervisory visits to Districts/peripheral labs o Training of all the DTO/STLS and LTs; o Proficiency testing of STLS o Data analysis and compilation for the State.
District Level Lab	o DTO Supervisory visits to TU and DMCs o Refresher training of STLS/LTs

Structural Level	Responsibilities
	o RBRC: Blinding and cross checking and reporting district level results to higher laboratories. o Preparation and distribution of reagents o Operationalise RBRC in the district o Supervise the STLS OSE of the DMCs.
Designated Microscopy Centre	o Providing the TB diagnostic services- sputum smear microscopy.

Table 3 : EQA functional components (adopted from ref 4, 5,8)

Particulars	OSE	RBRC	Panel Testing (proficiency testing)
Description	Periodic Supervisory visits to DMC/DTC/ STDC carried out within the RNTCP network in a systematic manner with the help of check-lists for identifying errors in sputum smear microscopy for immediate problem solving, corrective action, and on-site retraining.	A process of re-reading, in a higher-level lab, a statistically valid sample of slides from DMCs where routine TB diagnosis is performed.	A process by which a higher-level laboratory conducts proficiency testing of lab personnel of lower level lab with help of a set of unstained panel test slides. It checks the laboratory's staining procedure as well as the ability of the technician to recognize and quantitate acid-fast bacilli. The panel consists of five unstained smears manufactured and validated at the higher-level lab. A panel consists of a range of positives and least one negative AFB slide.
Procedure	NRL perform OSE of State level labs (STDCs). STDCs conduct OSE of the District level labs and District level labs supervise the TU and Designated microscopy centers. The visit includes a comprehensive assessment of laboratory organization,	A statistically valid sample of slides are obtained from the DMC and tested at district level lab by proficient technicians (controllers) for smear quality, grading and reporting. Slides are selected in a systematic random fashion and are blinded/ concealed to the identity of the technician who prepared the slides.	NRL conduct Panel testing of lab personnel of State level labs during their annual OSE. State level labs perform the panel testing of all the STLS in the district during their periodic OSE. Errors are validated as per table 6 and errors/ deficiencies are corrected on the site.

Particulars	OSE	RBRC	Panel Testing (proficiency testing)
	equipment, adequacy of supplies, reagent quality, SOPs, grading & reporting of AFB smear microscopy andinfection control measures.		
Advantages	1. It involves direct contact between peripheral (DMC) technicians and supervisory staff from the intermediate (STDC) or central level (NRL) thus motivates the staff. 2. Assessment of the laboratory under actual working conditions allows corrective actions to be implemented without delay.	Reflects a true picture of performance of laboratories offering routine diagnostic services at the peripheral level. Controlling laboratory can check not only the grading of the smear, but also the performance of the stain, the size of the smear, and the quality of the specimen – which influence the reliability of the final result	1. Provides a rapid picture of the proficiency of laboratories in state (or district). Distribution of the same panel to different laboratories will identify sites most in need of improvement. 2. Cost effective and easy to administer
Disadvantage	Consume significant resources – in travel costs as well as personnel.	Resources need to be mobilized to conduct the rechecking at higher-level lab.	1. Results might not reflect true picture of routine lab performance. 2. Technically intensive and heavy workload to higher-level labs.

Table 4: On-Site Evaluation of District Level Laboratory by State level laboratory (IRL)-Checklist

Topics for evaluation
1. Information about the laboratory administration and management
2. Action required as per the previous visit if this is not the first visit
3. Current visit details: ● Infrastructure (lab/power/water/microscope) ● Standard Operating Procedure ● Quality of staining regents ● Adequate stock and supply of reagents/supplies ● Disposal of infected material ● Internal Quality Control ● External quality control
4. DTC-EQA activities : ● Onsite Panel testing (table 3) ●● Assessment of STLS EQA responsibilities
5. Random Blinded re-Checking Results
6. Summary recommendation & Corrective actions undertaken as per the suggested recommendations.

Table 5: EQA On-site Evaluation of IRL by NRL - Operational and technical elements

a. Operational aspects

EQA-Laboratory organization:

- o STDC has separate infra-structure and building
- o STDC-EQA laboratory is independent of DTC-DMC
- o Head of IRL/ STDC Director works as an independent officer under State TB Control officer
- o STDC Director has no additional responsibility as the head of peripheral lab i.e., DTO of DTC
- o Vacant posts of Microbiologist and LTs are filled by STDC
- o STDC Microbiologist/ Pathologist is trained in RNTCP smear microscopy and in revised EQA guidelines
- o EQA team identified by STO/STDC for EQA training at NRL is posted at STDC after the training
- o Clinical functions are segregated from STDC
- o STDC to carryout Monitoring, Training, Culture & Sensitivity, Operational Research and Supervisory role for the State TB programme
- o EQA Training of all STLS & DTO completed by STDC with in a time frame
- o STDC ensures training of all the DTO, STLS and LTs of state in revised EQA
- o STDC, State TB Officer and State health authorities ensure availability of the full-time contractual STLS (one per TU) in the state
- o Annual Maintenance Contract of binocular- microscopes is done for the entire state

EQA-Panel testing :

- Panel testing slides are manufactured at STDC. Head of STDC should make all necessary infrastructure (biosafety cabinet, chemicals, vortex etc) arrangements for the same.

EQA- Onsite evaluation :

- Onsite evaluation of DTCs by STDC within time
- OSE report prepared at the end of OSE and submitted to DTO. OSE should be done for 2 or 3 consecutive days.
- OSE: Plan of action / annual tour programme to DTC by STDC EQA-team is prepared and adhered. This should be communicated to DTO well in advance.

EQA- RBRC :

- Logistics of RBRC adapted by DTOs are reviewed for correctness by STDC e.g., RBRC sample size for each DMC, Slides boxes, EQA forms, separate microscopes and area for RBRC
- Blinding of slides for RBRC and crosschecking to be performed at DTC and slides to be obtained for this from each DMC by the respective STLS
- RBRC results of DTCs are obtained monthly (in 'Annexure E'(electronic form))by the STDC
- Major and minor Errors in RBRC reviewed by STDC for corrective actions
- RBRC results of each DMC (in 'Annexure C') to be available at DTC for review
- STLS should fill-in their DMC-OSE forms, in full, with regard to Operational and technical problems. Suggestive actions should reflect the same.
- DTOs send feedback on RBRC results (in 'annexure D') to Medical Officers of DMCs.
- STDC should obtain and review, in time, 'action- taken-report' by the DTO on the OSE- corrective suggestions.
- Director STDC should send Action-taken-report on NRL OSE recommendations to Director, NRL within one month

Bio-waste management:

- Burying the waste in waste disposal pit at STDC/ DTC/DMC is practiced as per the recommendations of CTD. Burning of plastic is prohibited

b. Technical aspect

QA- IQC measures :

- Staining reagents are prepared at STDC
- Staining reagents are prepared at DTC/TU (not purchased from private manufacturer)
- Basic fuchsin and Methylene blue potency correction factor applied for reagent preparation
- Quality control of reagents to be checked for each batch using Control Smears
- Control smears usage is documented in registers and slides stored till the supervision by higher level lab

EQA : Panel testing

- o Expertise in panel test smear manufacturing and checking validation for Consistency
- o Correct administration of panel testing
- o Panel testing results of STDC Microbiologist/ LTs, administered by NRL on OSE need to be devoid of errors

EQA : RBRC

- o Technical difficulties with controllers incorrectly interpreting the RBRC slides.
- o No errors in recording and reporting results into various forms

Bio-waste management:

- o Proper waste management measures by lab personnel.
- o 5% Phenol solution (or effective concentration of 5% phenolic compounds) should be used at STDC/DMCs as disinfectant

Table 6: Annual sample size for RBRC for India (reproduced from ref 4)

Number of negative slides in the DMC in a year	Slide positivity rate (SPR%)				
	2.5-4.9	5.0-7.49	7.5-9.9	10-14.9	≥15
	Annual sample size of both positive and negative slides (Monthly sample size in parenthesis)				
301-500	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)

Table 7: Validation table for smear microscopy results- classification of error types (reproduced from reference 4 and 5)

LT results	Controller results				
	Negative	Scanty	1+	2+	3+
Negative	Correct	**LFN**	**HFN**	**HFN**	**HFN**
Scanty	**LFP**	Correct	Correct	**QE**	**QE**
1+	**HFP**	Correct	Correct	Correct	**QE**
2+	**HFP**	**QE**	Correct	Correct	Correct
3+	**HFP**	**QE**	**QE**	Correct	Correct

HFP: High false positive; **HFN**: High false negative; **LFP**: Low False Positive; **LFN:** Low False negative; **QE**: quantification error. **Correct:** results where the LTs results are in complete agreement with that of controller given the scope of smear microscopy performance.

Table 8 : Frequent causes for errors in RBRC and suggestive corrective measures

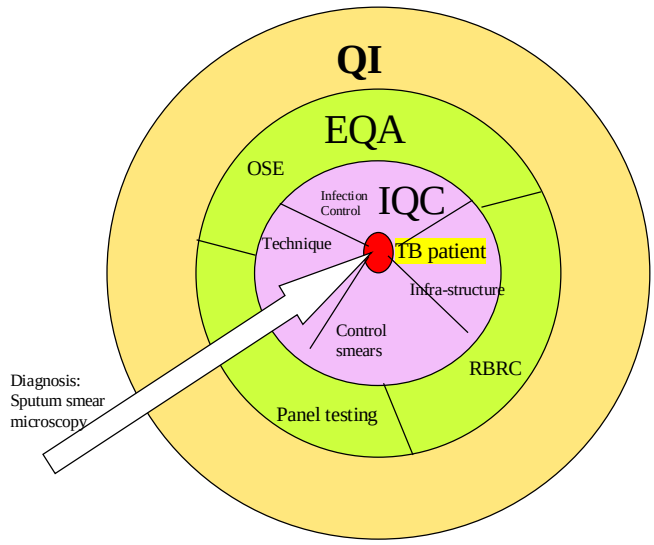
Error	Possible reasons	Corrective measure
False Positive	i) "Mucks"/stain deposits read as Acid-fast Bacilli ii) Immersion oil applicator touched the 3+ positive slides and AFB carried over to next slide iii) Fading of the stained AFB leading to first controller based errors. iv) Not cleaning the oil immersion lens after examination of a positive slide.	(i) Refresher smear & staining course for the LT (ii) Re-stain all the discordant slides before 2nd controller cross checks.
False Negative	i) Insufficient time spent for reading /read overlapping microscopy fields ii) Incorrect microscopy technique / defective microscope (e.g., x100 lens, microscope slide stage, condenser not raised fully) iii) Problems in staining (over de-colorisation, dull contrast) in potency calculation of staining reagents, out-of-date expiry staining reagents, insufficient staining times etc.,	i) Refresher smear & staining course for the LT ii) Evaluate the working of microscope with known positive and negatives, on-site. iii) Prepare new staining reagents, batch validate with control smears and re-stain and check the slides.
Quantification error	i) LT does not know how to grade the slide ii) A Smear scanned in less than 5 min. iii) Defective microscope	i) Refresh the technique of LT for staining and grading. ii) Evaluate the working of microscope with known positive and negatives.

Table 9 : Prominent Features of Revised EQA guidelines

- Identifying DMCs with performance problems—not intended to identify individual Technician errors or Validating patient diagnosis
- Emphasizes the need for onsite evaluation of laboratories using standard check-list
- Emphasizes the need for well-equipped STDC at State level.
- Panel testing as a method of EQA for proficiency of STLS
- Emphasis on random blinded Rechecking. Rechecking is always blinded
- Responsibility of RBRC vested with District TB officers at DTCs.
- Emphasis on the smallest possible sample size (LQAS) that provides sufficient reliable information on laboratory performance. For this all errors regardless of their level-High false errors (HFN & HFP) and Low false errors (LFN, LFP and QE)-are included.
- A second controller should resolve discrepancies after re-staining.
- Provides information to measure performance—based on the number and types of errors
- On-site rechecking of 5 positive and 5 negative slides picked up from the lab register in a systematic unblinded fashion is emphasized.
- Positive and negative slides are not sorted separately. Slides are arranged as per the lab register.
- Performance is not assessed based calculating a percentage of errors.

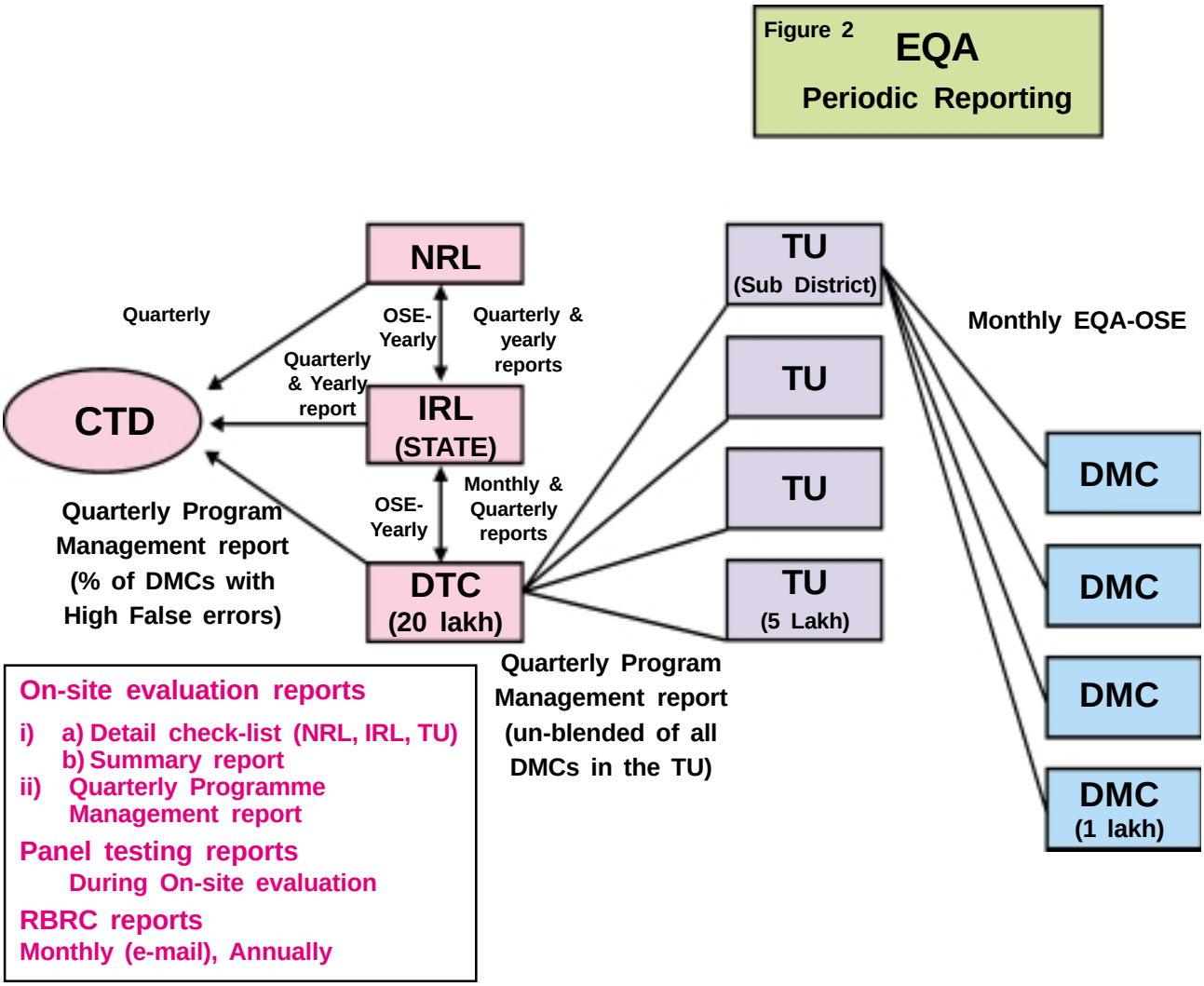
Sampling 10% of negatives and 100% of positives is not recommended.

Figure 1: Components of Quality Assurance (QA) System help accurate detection of TB Patient.



IQC-Internal Quality Control; **EQA**-External Quality Assessment; **QI**-Quality Improvement; **OSE**- Onsite Evaluation; **RBRC**-Random Blinded Rechecking of Routine Slides.

Figure 2



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