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Efforts of NTI as a national reference laboratory for implementation of External Quality Assessment for sputum microscopy in the ten allotted states during 2005-06

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Summary

Tools of External quality assessment such as (a) on-site evaluation (b) panel testing of the laboratory supervisors and (c) Random blinded re examination (RBRC) of the routinly examined sputum slides are used for finding and correcting the quality of sputum microscopy. Failure to maintain the quality would result in loss of credibility and waste of precious resources and inaccurate data and poor performance of the programme. The efforts of National Tuberculosis Institute as a National Reference Laboratory for implementation of EQA in ten allotted states for 2005-06 are reported in this article.

A total of 71 IRL laboratory personnel (37 Medical officers/ Microbiologists and 34 Sr. LTs) of 27 States were trained for five days on conducting the onsite evaluation (OSE), proficiency testing, manufacturing and validation of the panel testing slides, analyzing the RBRC data, and identifying & correcting the reasons for false results. Annual NRL-OSE visits to allotted ten states were undertaken during the period. Analysis of NRL-OSE recommendations revealed that Operational and managerial aspects (84%) outnumbered the proficiency in technical skills of the supervisory staff (16%). Comparative assessment of panel testing of intermediate reference laboratory (IRL) personnel and senior tuberculosis laboratory supervisor (STLS) in the districts visited by NRL indicated the effectiveness of manufactured panel slides in identifying the errors among STLS. RBRC coding & blinding registers, cross-checking rosters, and correct procedure of re-checking were established at district level in year 2005. The effective utilization of RBRC data, at district level, in correcting the systematic or technical problems of quality in sputum smear microscopy was ascertained during the NRL-OSE (district) visits of year 2006. Annual (year 2005) negative slide volume (ANSV) and slide positivity range (SPR) cross-tabulation at DMC level is reviewed for 4737 DMCs out of total 5747 DMCs monitored by NTI.

Based on the NRLs EQA-OSE recommendations and their implementation status in the states, Quality Improvement (QI) workshops/orientation meetings were conducted at National TB institute, Bangalore for the state level programme managers. During the workshop, a ready-referral list of common problems in smear microscopy quality assurance and their probable causes, possible solutions, prioritization of activities and plan-of-actions were compiled into a document.

Keywords : Revised National Tuberculosis Control Programme **,** Sputum Microscopy, External Quality Assessment

Abbreviations :

TB- Tuberculosis**; RNTCP-**Revised national tuberculosis control programme**; CTD-**Central TB division**; STDC-** State TB Training and

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Demonstration centers**; MoH-** Ministry of Health and family welfare**; DGHS-**Directorate general of health services**; WHO-**world health organization**; DOTS-**directly observed treatment, short-course chemotherapy**; NRL-** national reference laboratory**; IRL-**intermediate (state) reference laboratory**; DTC-**District tuberculosis centers**; TU-**tuberculosis units**; DMC-** Designated microscopy center**; EQA-**External quality assessment**; QA-**Quality Assurance**; QC-**Quality control (internal); **QI-**Quality Improvement**; OSE-**On-site evaluation**; ANSV-**Annual negative slide volume**; SPR-**slide positivity rate**; RBRC-**Random blinded rechecking**; STLS-**Senior Tuberculosis Laboratory supervisor**; LT-**Laboratory Technician**; ZN staining-** Zeihl-Neelsen staining**; NTI-**National tuberculosis Institute**,** Bangalore**; TRC-**Tuberculosis Research Center, Chennai**; LRS Institute-** Lala Ram Swarup Institute of TB & respiratory diseases**; DTOs-** District tuberculosis Officers**; STOs-**State Tuberculosis Officers; **HRD**-Human resource development ; **PT**-Panal testing;

Providing the good quality laboratory services for diagnosis and follow-up of treatment is one of the components of the RNTCP under DOTS[1,2]. Quality assurance programme monitors and evaluates, in a planned and systematic fashion, all functions of laboratory services from the TB suspect/patient registering for sputum examination, standardized testing procedures, infection control measures, providing correct and timely test results, to possible enrolment of the patient for treatment[3]. QA minimizes the false positive and false negative results in the laboratory services. Failures to maintain the quality would result in loss of credibility and waste of precious resources, at individual level, and inaccurate data and poor performance of the programme, in terms of case detection, at the national level[4,5]. Quality assurance components such as- quality control bench-marks, external quality assessment tools and overall quality improvement measures have been introduced based on international guidelines as well as experiences gained in India under the national control programmes[3,6].

The efforts of National Tuberculosis Institute as a National Reference Laboratory for implementation of EQA in the ten allotted states for 2005-06 are reported in this article.

I. Role of EQA in improving the quality of laboratory services :

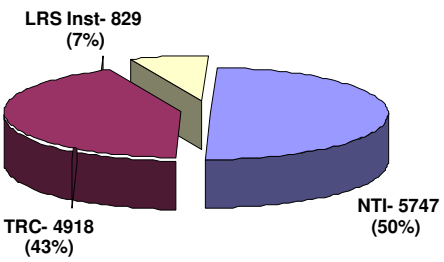
External quality assessment tools such as (a) on-site evaluation (b) panel testing or proficiency testing of the laboratory supervisors, and (c) Random blinded reexamination of the routine DMCs slides are used for finding and correcting the quality of laboratory services[3]. EQA helps in implementing the uniform standards in the TB diagnosis among the laboratories throughout the country. EQA acts as a tool to identify the root causes of the problems and suggests the corrective measures. EQA would also benefit the programme as a cost-effective tool by undertaking the remedial measures to avoid the recurrence of the problems. At the national level, EQA is necessary to ascertain the quality of data with regard to case-detection and cure-rate, and in the long-run enhances the credibility of the programme. Nation-wide consolidated and analyzed base-line EQA data would help the programme managers, as a quality indicator, in re-distribution of resources and effective management of the control programme. Effective EQA on-site evaluation visits are a motivating factor for Laboratory technicians and supervisory staff for on-the-site problem solving at DMC level, as well as sustaining the achievements of RNTCP at national level[2,4].

II. Lead role of Control Authorities (MoH/ DGHS-CTD) for assuring the quality of laboratory services

Central TB division (CTD), under the Min. of Health and Family welfare, is the national control

authority for implementing the RNTCP in the country. Given the prominent role of EQA in good quality diagnosis, CTD has initiated a number of steps since the inception of the programme. A RNTCP Laboratory consultative committee composed of laboratory experts from premier national TB institutions, WHO experts, and Central TB division was constituted and review meetings were organized in a quarterly fashion[8]. A national laboratory network committee was constituted consisting of all the laboratory experts from states and central institutions. Human resources for conducting the EQA were strengthened[1]. A revised national EQA guideline document was developed keeping in view the national and international guidelines[6]. Keeping in view, the enormous geographical natures of the country, three laboratories in the country, namely National Tuberculosis Institute, Bangalore, Tuberculosis Research Center, Chennai and Lala Ram Sarup Institute of TB & Respiratory Diseases, New Delhi, were recognized as the national reference laboratories[8]. A number of states were assigned for each NRL for QA/EQA implementation. For direct supervision, ten states and one union territory, were assigned to NTI. Like-wise, thirteen states including union territories were assigned to TRC. Eleven states, were assigned to LRS Institute. In effect, through state level and district level laboratories as the components of hierarchy, supervision of 50% (5747) DMCs are under NTI , 43% DMCs (4918) are under TRC and 7% DMCs (829) are under Lala Ram Sarup Institute (figure 1)[8].

Figure 1: Distribution of DMCs among NRLs*



NRLs are entrusted with responsibilities to develop technical expertise in EQA; Training of State Laboratory (IRL) staff in EQA; annual On-site evaluation visits of IRLs as per EQA guidelines including the assessment of quality of district OSE by IRLs; Proficiency testing of IRL laboratory personnel and review of IRL panel testing results of STLS; comprehensive review of RBRC activities/data from the districts; periodic reporting on EQA to CTD including the assessment of action-taken-reports submitted by the IRLs for NRL-OSE; and provide technical assistance on extent of EQA implementation to CTD during the RNTCP laboratory consultative committee meetings[2].

Periodic meetings of RNTCP laboratory consultative committee, with the full participation of NRLs and WHO experts, have strengthened the quality assurance aspects of laboratory. Some of the important technical decisions taken are listed in table 1.

III. Fulfilling the NRL responsibilities: Task assigned / Targets achieved by NTI (Year 2005-2006)

1. Training in External quality Assessment :

a. Starting from March 2005 to September 2006, total of 71 STDC/ IRL laboratory personnel (37 Microbiologists/ EQA Medical officers and 34 Sr. LTs) of 27 States were trained in NTI. The training was for five days each, focusing on conducting the Onsite evaluation (OSE) of district level labs, proficiency testing of Sr. Lab supervisors of the state, manufacturing and validation of the panel testing slides as per N-Acetyl L-Cystine procedure, supervising the RBRC procedure, assessment of RBRC data, and identifying and correcting the reasons for false results, and periodic reporting to NRL and CTD. Keeping in view the changes/transfers in the staff positions at the IRLs, trainings are conducted as and when required.

b. Training on Revised EQA guidelines for the DTOs, STLS at IRLs; All the ten states allotted to NTI, conducted state-level training of DTOs and STLS in performing and reporting On-site evaluation of DMCs and RBRC.

c. Sensitization / Orientation of Lab technicians at the DTCs; DTOs with the help of their respective WHO consultants and STLS orientated the Lab technicians of the peripheral diagnostic centers on internal quality control measures and maintenance of registers and records.

2. Annual NRL OSE visits : NTI conducted annual NRL-OSE visits to allotted ten states for year 2005. For the year 2006, six NRL-OSE visits were completed by 3rd quarter 2006. In total, 16 districts were visited by NRL team during the year 2005 and 13 districts in 2006 for assessment of IRL-EQA teams' OSE responsibilities. The summary recommendations of NRL-OSE visits in 2005 focused on operational problems in strengthening of the labs and staff in conducting the effective OSE visits to districts/ diagnosis centers, panel testing of all the STLS and operationalization of RBRC procedures/ facilities and reporting of results. The OSE visits for the year 2006 focused on effectiveness of recommendations of IRLs, and STLS-OSE, in identifying and correcting the problems related to quality assurance. More emphasis is given on the RBRC data validations and correcting the system whenever there were errors in the RBRC. Analysis of NRL-OSE recommendations (table 2) revealed that Operational and managerial aspects (84%) outnumbered the proficiency in technical skills of the supervisory staff (16%). Action-taken-reports submitted by IRLs, within one-month, of NRL-OSEs visits indicated effective implementation of corrective suggestions- HRD (81%), RBRC (82%), OSE (71%), and Panel testing (40%). In short, benefits to RNTCP as a result of NRL-OSE visits were (a) New IRLs were established /Infrastructure of existing IRLs strengthened (b) HRD of the IRLs strengthened (c) IRLs staff stopped conducing the patient care & clinical activities (d) the staff was oriented for their role in EQA supervision (e) panel testing of IRL supervisory staff and STLS carried out (f) RBRC procedure, and infrastructure was put in place (g) OSE and RBRC reports/analysis documented at IRLs.

3. Annual IRL OSE visits : IRL-OSE visits focused on correcting the problems identified by STLS-OSE checklists, including the un-blinded cross-checking of slides, and establishing the RBRC procedure and facility. For checking the quality of reagents, quality control slides usage and validation procedures were initiated and implemented in the district or TU level, wherever the reagents were prepared. RBRC blinding and cross-checking registers were started and maintained at district level. Monthly, e-mail based reporting of the RBRC errors for effective monitoring by state and central levels supervisors was initiated. Wherever appropriate, re-trainings and orientations for the staff lacking in the technical skills were conducted.

4. Panel testing results of IRL personnel and STLS : Comparative assessment of panel testing of IRL personnel and STLS conducted (of the districts visited by NRL) during the NRL-OSE visits indicated the effectiveness of manufactured panel slides in identifying the errors among the STLS (table 3 & 4). For the year 2005, errors in panel testing of IRL personnel (Microbiologist and Sr. LTs) were minimal at 96.90% sensitivity, 100% specificity compared to peripheral laboratory supervisors (STLS) at 78.04% sensitivity, 96.55% specificity.

5. RBRC activities at state level and district levels & reasons for the high false errors & corrective measures taken to improve the quality : NRL OSEs, in the first year of revised

EQA implementation, set priority for complete blinding, rechecking and correct and timely reporting of the data. RBRC coding & blinding registers, cross-checking rosters, and correct procedure of re-checking were established at district level. The effectiveness of RBRC data, at district level, in correcting the systematic or technical problems of quality in sputum smear microscopy was ascertained during the NRL-OSE (district) visits of year 2006. The districts were assessed on their ability to report errors, identify the probable causes, and implement the appropriate solutions. RBRC data was validated based on indicators such as annual negative slide volumes, slide positivity rates, discordance in re-checking among controllers and errors type, volume and at the DMCs. The data in the first year of the revised EQA implementation is being analyzed. The State level data of Maharashtra indicated that while some of the districts were completely devoid of errors, others reported high errors. For the year 2005, there were 0.16% high false errors (209 slides) among the total RBRC slides (1,34,496 slides) re-checked by the controller at district level. The minor errors (Low false errors and quantifications errors) were 0.33% (440 slides) of total slides rechecked. The IRL-OSE reports of Maharashtra indicate that the reasons for high false errors in the DMCs were investigated and corrective measures were initiated by the concerned District TB officers.

6. EQA -Annual negative slide volume (ANSV) and Slide positivity range (SPR) cross-tab at DMC level for the year 2005: Compiled data ANSV and SPR for the 9 state (Jharkhand, Madhya Pradesh, Maharashtra, J&K, Orissa, Karnataka, Rajasthan, Pondicherry and Maharashtra) allotted to NTI is given in Table 5. Bihar did not provide the data. The analysis of the data from this table indicates that;

a. Majority of the DMCs (82%) were having the ANSV and SPR within the accepted range. ANSV of >500 slides and annual SPR of ≥ 5% indicate the acceptable quality criteria for RNTCP demographic calculations of work-load of TB suspected in a DMC.

b. The DMCs with low SPR (<5%) & low ANSV (<300) contribute to 2.9% (138) of the total DMCs (4725) in eight states in the year 2005. Among these, 57.35% DMCs (78) were situated in the state of Madhya Pradesh.

c. DMCs with ANSV of >1000 but SPR of <5% accounted to 3.7% (174) of the total DMCs. Among these DMCs, 37.57% (65) were situated in the state of Maharashtra.

d. DMCs with ANSV of <300 but SPR of >15% accounted to 2.7% (127) of the total DMCs. Among these DMCs, 35.43%(45) were situated in the state of Madhya Pradesh.

IV. Quality Improvement workshops/EQA orientations to the state level programme managers in implementation of EQA activities:

Based on the NRLs EQA-OSE recommendations and their implementation status in the states, Quality Improvement (QI) work-shops/orientation meetings were conducted at National TB institute, Bangalore for the state level programme managers (STOs and STDC/IRL directors). Four batches of such national level workshops were conducted, each for 2 days duration, for 31 states/UTs of the country during Dec 2005 & March 2006. Total of 54 STOs and directors STDCs/IRLs, participated in these workshops. Interactive group-discussions in these meetings focused on a range of state level EQA issues such as setting up of IRLs, human resources, and conducting effective OSE by IRL

staff, to DMC level operational/technical issues such as high annual negative slide volumes & low slide positivity rates, quality specifications for the microscopy staining reagents and safe disinfection/disposal of infected waste. An exhaustive list of common problems in smear microscopy quality assurance and their probable causes, possible solutions, prioritization of activities and plan-of-actions (of all batches) were compiled into a ready-reference document. As a result of these meetings it was emphasized by all TB programme managers that 94% of the EQA implementation problems occurred at the IRL/State level and could be resolved at the state level.

V. Perspectives :

There is an important supervisory and technical guidance role for national reference labs in QA & EQA of sputum smear microscopy laboratory network in the RNTCP. EQA aims at constant problem identifications, exploration of causes, suggestions and implementation of the corrective actions for good quality laboratory services. In the initial phase of EQA implementation significant progress has been achieved. These efforts need to be sustained and improved as per the needs and lacunae of the laboratories. The lab committee would be assuming the paramount importance in consolidating the data of all three national reference laboratories and guiding the programme manager in the states on utilizing and providing the Quality assured diagnostic services to the Tuberculosis suspects and patients. In addition to implementation of effective EQA network in the country, national reference laboratories would be equipping themselves for helping the national control authorities towards pursuing high-quality DOTS expansion and other components outlined in the "stop TB strategy" by the WHO and agreed by the national/ international health community[7].

Table 1: Some important QA/QC measures discussed/ recommended by the RNTCP laboratory consultative committee[8]

Sl.No.	Technical issues	AQ/AC measure recommended
1.	Disinfection: 5% freshly prepared bleach, or 10% commercially available bleach	5% Phenolic solution to replace bleach as disinfectant
2.	Disposal of the examined slides by breakage/ disinfection of slides	Disposal of the examined slides is to be done by chemical disinfection and burying. Breakage of slides is to be discontinued.
3.	Disposal of infected waste	Disposal of infected materials to be done as per RNTCP bio-waste management procedures. Burning of plastic is to be discontinued and burying of the waste is advised.
4.	Control slides for checking the quality of prepared reagents	One positive (3+) slide and one Negative unstained slide to be used-usage to be documented

Sl.No.	Technical issues	AQ/AC measure recommended
5.	Immersion oil/ cedar wood oil	Liquid paraffin 'heavy' with specifications mentioned in EQA document should be used. Use of cedar-wood oil to be stopped.
6.	Method for removal of immersion oil	Xylene or Xylol should not be used for removal of oil. Inverting the examined slides on soft tissue paper is recommended.
7.	STLS module/manual	Revised STLS module and LT module were developed incorporating the EQA needs.
8.	Maintaining the proficiency of newly trained STLS who are to participate in RBRC as controllers	To observe at least 10 slides in a routine manner each day at their work-places till they achieve the proficiency
9.	Specifications for ZN staining chemicals	Developed and incorporated in the EQA document.
10.	Xylene /xylol for cleaning the lenses (objective and eyepiece) and removal of immersion oil	Xylene /xylol are not used for microscopy purposes since it makes the objectives of microscopes non-usable.
11.	Grading of positive slides	For 'scanty' positive only 100 fields are sufficient
12.	Re-examination for sputum negatives	For 3 sputum negatives with clinical symptoms of TB are advised to under go re-examination (RE) for diagnosis after administration of 10-14 days of non-TB drugs.
13.	Preparation of reagents by lab staff / not to use ready made reagents	STLS are entrusted with preparation of laboratory reagents.
14.	Distilled water/de-ionized or de-mineralized water	Tap water not to be used for reagents preparation, only distilled water or de-ionized or de-mineralized is to be used.
15.	Potency of ZN staining chemicals	Potency correction factor, wherever needed, is to be applied during ZN staining reagents preparation.
16.	Expiry date for ZN staining reagents	Expiry date for the ZN staining reagents is four months from the date of preparation.

Sl.No.	Technical issues	AQ/AC measure recommended
17.	Weighing balance	Districts are allowed to procure the weighing balances, preferably electronic
18.	RBRC coding & blinding register and re-checking rosters	RBRC coding & blinding register and re-checking rosters are to be maintained in each DMC
19.	RBRC: Coding and blinding procedures for districts with only 2 TU	The RBRC should be conducted with the help of neighboring district. The STLS are to be interchanged or clubbed, RBRC slides are not be sent to other district.
20.	Reporting of RBRC annual state-wise data	The reporting format to include the % of DMCs with high false positive and high false negative results separately
21.	Separate Microscope for RBRC	A separate microscope is provided for each district for conducing RBRC
22.	Transport and storage of RBRC slides	Separate slide boxes are recommended for transportation, and storage of RBRC slides.Examined slides are to be stored for at least 2 months or till the RBRC data is sent to CTD/feed-back is provided to DMCs.
23.	Panel slides for proficiency testing	Both manufactured and patient-wise slides for panel are accepted, as long as they and able to identify errors of supervisors (STLS)

Table 2 NRL OSE recommendations & Implementation status in ten states

Group-wise	Total actions recommended	Actions implemented	Actions pending	% implementation
HRD	74	60 (81.08)	14	81.08%
RBRC	23	19 (82.61)	4	82.61%
OSE	28	20 (71.43)	8	71.43%
PT	25	10 (40.00)	15	40.00%
Total	**150**	**109**	**41**	**72.66%**

Table 3: NRL Panel testing of IRL microbiologists and LTs#.

IRL results	*NRL results†*	
	Positive	***Negative***
Positive	94	0
Negative	3 (LFN)	43
Total	97(96.9%)	43(100%)

Number of personnel tested=28

† Panel testing slides were manufactured by the N-acetyl L-cysteine method at the NRL. Statistically validated for consistency (±2SD). A test set of 5 slides were provided to each Microbiologist/Sr LT during the OSE visit. A standard time of 45 min was given for examination of five slides. The test is administered as per the EQA guideline document. All the discordant results were verified by the NRL Sr LTs before declaring the error.

Table 4: NRL Panel testing of STLS during sample DTC visits #.

STLSresults	**NRL results**	
	Positive	***Negative***
Positive	40	1 (HFP)
Negative	9 (2HFN & 7LFN)	20
Total	49 (78.04%)	21 (96.55%)

Number of personnel tested = 14

† Panel testing slides were manufactured by the N-acetyl L-cysteine method at the NRLs. Statistically validated for consistency (±2SD). A test set of 5 slides were provided to each STLS during the OSE visit. A standard time of 45 min was given for examination of five slides. The test is administered as per the EQA guideline document. All the discordant results were verified by the NRL Sr LTs before declaring the error.

Table 5: EQA -Annual negative slide volume (ANSV) and Slide positivity range (SPR) at DMC level for the year 2005 (for ten NTI states)

ANSV	**Number of DMCs**			**Total DMCs**
	Slide positivity rate			
	<5 %	**5% to 15%**	**>15%**	
≤ 300	138(2.91%)	177(3.74%)	127(2.68%)	442(9.33%)
301-500	83(1.75%)	251(5.3%)	135(2.89%)	469(9.9%)
501-1000	161(3.4%)	700(14.78%)	314(6.63%)	1175(24.8%)
> 1000	174(3.67%)	1920(40.53%)	557(11.76%)	2651(55.96%)
Total DMCs	556(11.74%)	3042 (64.22%)	1133(23.92%)	4737(100%)

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