

## Qualitative & quantitative assessment of ten Intermediate Reference Laboratories for quality of sputum smear microscopy based on RNTCP on-site evaluation

*T Ajaykumar\**, *S Shyni †*, *S Shiju †*, *VH Balasangameshwara‡* & *P Kumar ø*

### Abstract

The paper reports the qualitative and quantitative assessment of the NTI OSE check-lists for the years 2005 and 2006 of ten state-level Intermediate Reference laboratories (IRLs) to establish effective supervision for quality of sputum smear microscopy. The assessment also indicates the effectiveness of NTI-OSEs. Quality related problems were identified, causes explored and solutions provided for establishing quality sputum microscopy network. NRL OSE reports, IRL OSE reports, RBRC records and action-taken-reports formed the material for assessment.

Overall, 44.15% errors in laboratory checklist-items were identified in ten IRLs during first year OSE (2005). Majority of errors occurred in EQA (92.6%), internal quality control (90%), staining reagents / equipment (56.6%), infrastructure (42%), and bio-safety practices (40%). Nine IRLs were performing clinical activities. Staffs were not adequate in IRLs. As a result of OSEs, IRLs discontinued the clinical activities. OSE based supervision of district labs by IRLs, ranged from 58.3% to 100%. Overall improvement for laboratory check-list items, between two annual OSEs years (2005 and 2006), in five states, was 77.9%. IRLs were strengthened for staff, facilities and OSE functions. NTI OSE recommendations improved

IRLs for planning & prioritization of their OSE visits, ensuring complete blinding for RBRC, taking corrective measures for high false errors and monitoring implementation of recommendations. OSE and RBRC supervision at District level needed regular monitoring. NTI efforts strengthened the feed-back mechanism for quality improvement among Lab network partners.

**Abbreviations & Key words:** External quality assessment (EQA); On-site evaluation (OSE); Check-list; National reference laboratory (NRL); Intermediate reference laboratory (IRL); District Tuberculosis Center (DTC); Designated Microscopy center (DMC); Revised National Tuberculosis Control Programme (RNTCP); Random blinded Rechecking (RBRC); Human resources (HR); Laboratory Technician (LT)

### Introduction

Providing the good quality sputum microscopy laboratory services for diagnosis and follow-up of treatment is a key component of the Revised National Tuberculosis Control Programme (RNTCP) in India.<sup>1</sup> Quality assurance system for the sputum microscopy is aimed at minimizing the false positive and false negatives results.<sup>2</sup> Quality assurance system with its components- quality control bench-marks, external quality assessment tools and overall quality improvement measures, were introduced

\* Consultant Microbiologist, †Sr Laboratory Staff, ‡CMO (NFSG) & I/c Bacteriology Section, ø Director and corresponding author, National Tuberculosis Institute, Directorate General of Health Services, Government of India, 8, Bellary Road, Bangalore-560003.

in India in 2005 adopting international guidelines.<sup>2,3,4</sup>

Sputum microscopy laboratory network involves national level reference laboratories (NRLs), state level intermediate reference laboratories (IRLs) and peripheral laboratories at district (DTC) and designated microscopy centers (DMCs. External Quality Assessment (EQA) tools such as (a) on-site evaluation (OSE) (b) panel testing or proficiency testing of the laboratory supervisors, and (c) Random blinded re-checking (RBRC) of the routine DMCs slides are employed for identifying and correcting poor laboratory practices and procedures.<sup>3</sup>

RNTCP has recognized three laboratories in the country, National tuberculosis Institute, Tuberculosis Research center, and Lala Ram Sarup Institute of TB & Respiratory Diseases as National Reference Laboratories in the EQA network. NRLs were assigned the task of developing & strengthening IRLs. Ten states, mostly in northern India, were assigned to National Tuberculosis Institute.<sup>3</sup>

NRLs responsibilities with regard to IRLs include training, annual on-site evaluation, proficiency testing of personnel, quality assessment of OSEs conducted by IRLs, review of panel testing results of supervisors of DMCs, and review of RBRC activities. NRLs also provide technical assistance on EQA implementation during the RNTCP laboratory network meetings<sup>3</sup>. First round of NRL –OSE visits were undertaken to ten IRLs in 2005 and repeat visits were undertaken to five IRLs in 2006. The Objectives of this article are (i) To analyze the performance of ten Intermediate Reference Laboratories(IRL) in the year 2005; (ii) To assess the improvement in performance of IRLs in 2006 against the recommendations given during 2005 OSE visits.

## Materials & methods

On-site evaluation was defined as the check-list based assessment of the laboratories to identify the problems, explore probable causes and suggest appropriate corrective solutions.

Three sets of similar but separate OSE checklists were employed in RNTCP laboratory network for NRLs, IRLs and Peripheral labs<sup>3</sup>. The NRL-OSE of IRL was conducted after training of the IRL EQA team i.e.microbiologists and LTs, for five days. Three days were allotted for internalization of the guidelines, and a day for practical training on manufacture & validation of panel testing slides. One day of field visit to district lab to familiarize usage of checklist and prioritize suggesting recommendations. The pre and post -training test assessment indicated overall 54.1% improvement among 36 trainees (average of 25.73% pretest and 79.80% post-test marks) (data not shown).

NRL to IRL OSE checklist consisted of five sections (see annexure 1). Section I contained general information on IRL. Section II pertained to the action-required as per the previous annual OSE visit. Section III consisted of 35 laboratory checklist items divided into ten sub-heads. Section IV (a) pertained to the quality of panel slides prepared at STDC, and NRL panel testing results of IRL lab staff. Section IV (b) gave data on the extent of DTC-OSE and STLS panel testing. Section V reviewed IRL OSEs of DTCs.

NRL EQA team comprising of one microbiologist and two senior technicians performed the OSE of IRLs for three to four days employing the OSE checklist (annexure 1). One or more districts were visited for assessment of quality of IRL OSEs. Panel testing slides were prepared and batch validated. Single batch of panel slides were used for all states. A set of five slides, was used to panel test each IRL lab personnel. Discordant results were verified by

the NRL Sr. LTs before declaring the error.<sup>3</sup> Prioritizations of NRL visits to districts during second year OSE was based on the annual RBRC errors- District with high false errors, and/ or without false errors, for entire year, were visited.

Summary recommendations were submitted to the heads of the respective IRLs. State TB Officers presented selected recommendations to health directorate for solving operational problems. Action-taken-reports were obtained within one month of the visit.

Under RNTCP, RBRC is routinely carried out, monthly, at the district TB laboratory, based on the annual Lot Quality Assurance Sample (LQAS) size.<sup>3</sup> Smears of DMCs are picked up by randomized systematic sampling into slide boxes. DTO codes the slide boxes and exchanges the boxes among the controllers. Blinding and re-examination registers are maintained by DTO. First controller (STLS) re-examines for smear quality and results. DTO decodes the identity of LTs slide results and matches with controller's result. Discrepant smears are re-stained and examined by second controller. Errors are rectified by DTO giving priority to the DMCs with major errors. IRLs, annually, consolidate RBRC results and also supervise the correctness and completeness of RBRC procedure. The procedures and outcome of RBRC were evaluated during NRL visits to IRLs & respective DTCs.

First NRL OSE visits were carried out in year 2005 and followed up with second NRL OSE visits, in five states, in year 2006. Improvement between the annual visits was defined in terms of correction of total number of errors (items marked as 'No' for adequate and/acceptable column of the checklist) of the first OSE visit compared to the second. Data of OSE summary recommendations was

analyzed, segregated into subheads and most frequent recommendations were listed out.

## **Results**

### **I. Performance of ten Intermediate Reference Laboratories (IRL) in the year 2005**

The results of first year NRL EQA On-site evaluation of ten IRLs are presented, section-wise, below (see annexure I for check-list format).

#### **Section I : General information-Personnel & facilities :-**

Of the total ten IRLs, six (Rajasthan, West Bengal, Maharashtra, Jammu & Kashmir, Orissa, and Bihar) were headed by a Director or Superintendent, two IRLs (Karnataka and Madhya Pradesh) by State TB Control Officers, and remaining two IRLs (Pondicherry and Jharkhand) by Hospital superintendents. Building facility wise, three IRLs (Maharashtra, West Bengal, Orissa), were functioning from Medical colleges, another three (Madhya Pradesh, Jharkhand, Pondicherry) were located in TB Sanatoria. The remaining four (Jammu & Kashmir, Karnataka, Rajasthan and Bihar) were having independent building facility. Nine out of the ten IRLs (Bihar was exception) were also carrying out the clinical functions of patient care & management.

#### **Section II : Actions required as per previous visit: -**

Not applicable, since this was first ever evaluation EQA visit by NRL under RNTCP.

#### **Section III : Current visit particulars : Laboratory Checklist items :-**

The proportion of errors in Laboratory checklist items sub-head wise were EQA (92.6%), internal quality controls (90%), Staining reagents / equipment (56.6%), Infrastructure (42%), and Safety Practices (40%). Per cent of errors in

other sub-heads of check list were - disposal of infected material (36.6%), adequate stock and supply (27.7%), Standard Operating Procedure

(20%), Training status (18.18%) and Binocular Microscope (10%) (Table 1). Overall, errors were identified in 44.2% of laboratory checklist items in the ten states.

**Table 1: Number and Percent of Errors in each sub-head in ten IRLs for first year OSE**

Sr. No	Sub-head	Errors (%) Adequate / Acceptable * = "NO"	N = Total check-list items (100%)
1	External quality Assessment	38(92.6)	41
2	Internal Quality Control	9(90)	10
3	Staining reagents / equipment	34(56.6)	60
4	Infrastructure	21(42)	50
5	Safety Practices	4(40)	10
6	Disposal of infected material	11(36.6)	30
7	Adequate stock and supply	25(27.7)	90
8	Standard Operating Procedure	6(20)	30
9	Training status	2(18.18)	11
10	Binocular Microscope	1(10)	10
	<b>Total</b>	151(44.15)	342

\* sorted in descending order of errors (%)

All the ten IRLs had errors for checklist item 32 (whether all DTCs were visited, at least once, by IRL staff, as per their tour programme for the year), and item 23 (major equipment for manufacture of panel slides) (table 2). Errors in internal quality control slides (item 31) and all the items of external quality assessment (items 33-35) occurred in nine out of ten IRLs. Seven out of ten IRLs failed to include the potency correction factor for stains during preparation

(item 18 & 19). Ready-made commercial staining reagents were used in 3 IRLs. Weighing balances (item 22) were not available in four IRLs. In three IRLs, distilled water was not used (item 21) for stains. Seven IRLs were using the 5% hypochlorite/ commercial bleach for the disinfection (item no 14) instead of 5% phenolic solution recommended. Waste disposal (item 26) was by burning in four IRLs.

**Table 2 : Frequency of errors among individual checklist items in the first year OSE**

Sr. No	Checklist item numbers	Number of states with errors
1	23, 32	10
2	31,33,34,35	9
3	14,18,19	7
4	1	6
5	4, 25	5
6	3,16,22,26,28	4
7	2,5,8,10,17, 15,20,21	3
8	6,13,27, 21	2
9	7,9,11,12,24,29,30,36	1

IRL-wise analysis indicated that Jharkhand (85.2%), Bihar (55.8%), and Madhya Pradesh (55.8%) had maximum errors in the checklist

items, and Pondicherry (32.3%), West Bengal (26.4%), Maharashtra (25%) had minimum number of errors compared to other states (table 3).

**Table 3 : Overall number and percentage of Errors in ten states of NTI for year 2005**

Sl.No.	State	State Total check- list items (100%)	Errors (%)
			Adequate /Acceptable *= "NO"
1	<b>Jharkhand</b>	34	29 ( <b>85.29</b> )
2	Bihar	34	19 (55.88)
3	Madhya Pradesh	34	19 (55.88)
4	Orissa	34	15 (44.11)
5	Karnataka	34	14 (41.17)
6	Jammu & Kashmir	34	13 (38.23)
7	Rajasthan	34	13 (38.23)
8	Pondicherry	34	11 (32.35)
9	West Bengal	34	9 (26.47)
10	<b>Maharashtra</b>	36	9 ( <b>25</b> )
	<b>Total</b>	<b>342</b>	<b>151 (44.15)</b>

\* Sorted in descending order of errors (%)

#### **Section IV : Panel testing and OSE functions:-**

West Bengal and Maharashtra used the patient-wise unstained testing smears for panel testing. Rajasthan and Jammu & Kashmir used stained patient smears. Six IRLs were not carrying out panel testing of the STLS.

Twenty eight IRL laboratory personnel were panel tested during NRL OSE. In total, 140 (97 Positive & 43 Negative) unstained smears were used for testing. Three Low False negative errors (LFN)<sup>3</sup>- Karnataka (2) and Orissa (1)-were identified for one medical officer and two LTs (sensitivity 96.90% and specificity 100%). The errors occurred due to poor quality stains, and failure to read required number of microscopy fields.

By the end of 2005, five IRLs- Maharashtra, Karnataka, Rajasthan, West Bengal and Pondicherry had completed OSE of all districts in their respective states.

#### **Section V : Review of IRL OSE results of DTCs :-**

Eleven districts in ten states were visited during first NRL-OSEs. Effectiveness of training of staff, internal quality control measures, quality of STLS OSE, correct LQAS size and blinding & re-examination procedures for RBRC, reporting and correcting DMCs with the OSE and RBRC errors, were among the main recommendations provided (table 4)

**Table 4: NRL District visits: Recommendations**

---

<b>EQA-RBRC</b>	
1	Records to be maintained for coding, blinding of RBRC slides & reexamination roster
2	Discordant results and errors slides to be provided to DMCs with a request for action-taken-report for errors
3	Errors to be investigated: RBRC high false errors need to be investigated and corrective actions taken by the DTO by supervisory visit to the DMCs with such errors.
<b>EQA-OSE</b>	
4	Regular and periodic review of STLS OSE check-list to be done by DTO for the problems identified in DMC
<b>Staining reagents / equipment</b>	
5	False results would be reduced by including potency correction factor for preparation of stains. MSDS information to be made available on the chemical bottle
6	Ready made reagents/stains of (unknown specifications) to be discontinued
7	Working weighing balance to be made was available in the DTCs
<b>Internal Quality Control</b>	
8	Every new batch of reagent prepared to be quality checked with control smears, validated and results documented before distributing the reagents to DMCs
9	Reagents for smear microscopy were not prepared / stored according to RNTCP lab manual, date of preparation and concentration was not indicated on the labels of the reagents
10	Only 100 oil immersion microscopic fields need to be read for scanty positive grading instead of 200 fields.

- 11 Cedar wood oil to be discontinued as immersion oil for x100 lens
- 12 Xylene to be discontinued for cleaning microscope lens and removing the immersion oil
- 13 Lab Supervisors to prepare the stains
- 14 The smears to be arranged in the storage slide box as per the laboratory entries in the register

**Infrastructure-facilities**

- 15 Separate area (table and staining sink) and a dedicated binocular microscope for conducting RBRC to be ensured in each DTC

**Infrastructure-HR**

- 16 DTOs should not have additional responsibilities of general health services
- 17 Ensure filling of full time DTOs/STLSs/LTs posts in the peripheral laboratories
- 18 Lab Supervisors should not have additional duties of general health services
- 19 Lab supervisors not to performing the Lab Technicians routine work, which would compromise the supervisory responsibility

**Disposal of infected material**

- 20 Ensure that 5% phenol/ phenolic solution is used for disinfections of infected waste, at all DMCs in the state
- 21 Waste need to be disposed off by after chemical disinfection by burying

**Adequate stock and supply**

- 22 Sufficient number of slides boxes to be provided to each DMC for collection and storage of RBRC slides

**Training status**

- 23 Training to STLS for RBRC and unblinded cross-checking found inadequate
- 24 Recently appointed STLS were not trained in smear microscopy/module but were carrying out RBRC activities.

**Binocular Microscope**

- 25 Annual Maintenance Contract for service, repair and replace of parts for binocular microscope to be arranged at all DMCs of the state.

**SOP**

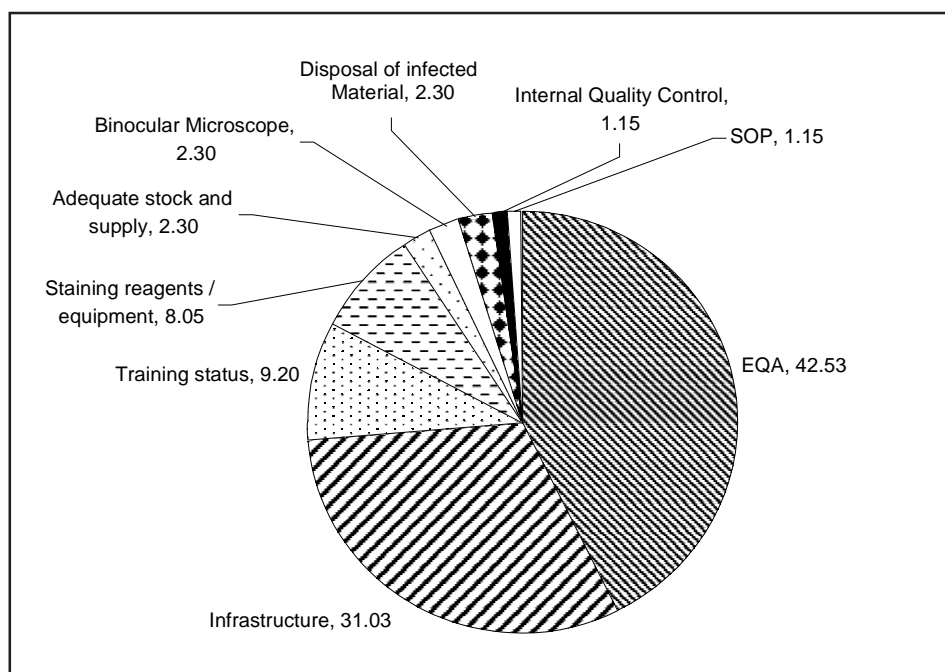
- 26 Displayed SOP for RNTCP smear staining procedure in the lab is not complete/displayed.

**OSE Summary recommendations for the first year :**

In the initial stages of EQA implementation in the states, several recommendations provided to the IRLs were similar. Among recommendations, total of 144, provided for ten

IRLs, 87 (60.4%) were repeated in two or more states. The distribution of recommendations among ten IRLs under the different sub-heads indicated that external quality assessment, infrastructure, and training requirements accounted for 42, 31, 14 per cent errors (Fig.1)

**Fig 1 : Distribution of actions recommended in ten states during OSE**



## II. Improvement in performance of IRLS In 2006

IRLS of Maharashtra, West Bengal, Karnataka, Rajasthan and Pondicherry were visited in the second year. A new location and building was provided to IRL Karnataka by Health services keeping in view of requirements.

Significantly, all the five IRLs stopped clinical functions. Three TB medical officers and five LTs were newly posted for EQA responsibilities in West Bengal, Rajasthan and Karnataka.

Overall, 40 out of 50 recommendations mentioned during the first OSE visit were implemented by IRLs (table 5-6).

**Table 5 : Actions recommended in the first NRL OSE and their implementation status as assessed during the second OSE**

State	Actions recommended in 2005 (100%)	Actions not completed (%)
West Bengal	10	3 (30)
Karnataka	11	2 (18.2)
Rajasthan	12	1 (8.3)
Pondicherry	11	1 (9.1)
Maharashtra	6	3 (50)
<b>Total</b>	<b>50</b>	<b>10(20)</b>



**Table 6 : Details of recommendations of first OSE visit identified to be not-implemented in second OSE\***

---

**External quality Assessment-OSE**

1. The STLS-OSE check list should be filled completely with recommendations for improvement
2. DTO and second MO should identify operational problems from STLS-OSE check list and take corrective measures

**External quality Assessment-RBRC**

3. All RBRC reports (electronic formats) should be analyzed by MO of STDC and feed backs given to DTOs

**Infrastructure-facilities**

4. The laboratory facilities needs to be expanded with additional space

**Infrastructure-HRD**

5. Microbiologist post and LTs posts needs to be filled up
6. Workload of the Laboratory Technician at DTC attached DMC was very high (approximate load of 100 slides per day). Additional Laboratory technician is required at DMC for improvement in quality

**Binocular Microscope**

7. AMC for Microscopes to be reviewed by State TB Cell, keeping in mind the observations made by DTOs
- 

\* *Three recommendations repeated*

Overall improvement for check-list items (section III) between the two annual OSE visits was 77.9% (table 7). In West Bengal five errors of first visit remained uncorrected and two new errors identified during second visit. Maharashtra had one new error and one uncorrected error. Pondicherry had one new error. Karnataka and

Rajasthan had no new errors; had three uncorrected errors. State-wise over-all improvement between two annual visits (table 8) indicated maximum improvement in Pondicherry (90.9%) compared to Karnataka (78.5%), Rajasthan (61.5%), Maharashtra (77.8%) and West Bengal (22.2%).

**Table 7 : Sub-head wise comparative assessment in laboratory check-list items between two annual OSE visits among five states**

Sub-head*	Number of items with Acceptable/Adequate= "NO"		Total check-list items	Over all improvement (%) between the visits
	First visit	Second visit		
Internal Quality Control	4(80)	0	5	100
External quality Assessment	17(85)	4(20)	20	76.5
Training status	6(60)	2(20)	10	66.7
Staining reagents / equipment	13(43.3)	5(16.6)	30	61.5
Infrastructure	10(40)	05(20)	25	50
Adequate stock and supply	7(15.5)	1(2.2)	45	85.7
Standard Operating Procedure	1(6.6)	0	15	100
Disposal of infected material	1(6.6)	1(6.6)	15	0
<b>Total</b>	<b>59(34.16)</b>	<b>13(8.69)</b>	<b>165</b>	<b>77.9</b>

\*The Sub-head of checklist: Binocular Microscope and Safety Practices (each sub-head had only one item) were acceptable/ adequate at STDC level for both year 2005 & 2006.

**Table 8: State-wise comparative assessment in laboratory checklist items between two annual OSE visits among five states**

State	First year*		Second year*		Per cent improvement (a-b)/ a*100
	No. Of items with errors (a)	Percent	No. Of items with errors (b)	Percent	
Karnataka	14	41.1	3	8.5	78.5
Pondicherry	11	32.3	1	2.8	90.9
Rajasthan	13	38.2	5	14.2	61.5
Maharashtra	9	25	2	5.7	77.8
West Bengal	9	26.4	7	20	22.2
<b>Total</b>	<b>56</b>		<b>18</b>		<b>67.8</b>

\* Total check list items, each state =34

# Improvement was measured in terms of % reduction in the errors in checklist items between first and second visits.

Three states were manufacturing the panel testing smears (Maharashtra, Karnataka, and Pondicherry). Two states were using the patient-wise panel testing smears (Rajasthan and West Bengal). Results of panel testing of the STLS indicated that errors committed by the STLS, ranged from 0% to 3.2% (data not shown).

Twenty one IRL laboratory personnel panel tested during second NRL OSE. In total, 110 (97 Positive & 43 Negative) unstained smears were used for testing. Three LFN (Rajasthan 2 and Maharashtra 1) and one HFN (Rajasthan) were identified for one medical officer and three LTs (sensitivity 94.90% and specificity 100%). Three errors occurred at Rajasthan because untrained LT stained the smears for all the other IRL lab personnel.

Completion of OSE responsibilities of IRLs to district labs (section IV b) data, at the time of second NRL-OSE visits, ranged from 100% to 58.3%. West Bengal and Pondicherry fully completed the OSE visits (expected visits in a

year) for the assessment period while Rajasthan (69.2%), Karnataka (66.6%), and Maharashtra (58.3%) had partially completed the visits compared to expected visits.

Overall, DTC-OSE recommendations of IRLs indicated Operational problems (87%) were more compared to technical improvements (13%) (data shown). The impact of these recommendations could not be assessed fully, at the time of NRL-OSE, due to lesser number of action-taken-reports submitted for the IRL-OSEs by the DTOs.

Number of summary actions recommended in the second OSE compared to the first indicated that, 10(14.4%) and 37 (53.4%) out of total 69 recommendations were provided for improvements in infrastructure and external quality assessment respectively. The data for same in the first year OSE were 20 (37%) and 24 (44.4%) out of the total 54 recommendations (table 9).

**Table 9 : Number and percent of summary recommendations provided during NRL OSE among five states during two annual OSE visits**

Sub head	First year (%)	Second year (%)
Adequate stock and supply	1 (1.84)	3 (4.3)
Binocular Microscope	1(1.84)	4 (5.7)
Disposal of infected material	1 (1.84)	4 (5.7)
<b>External Quality Assurance</b>		
External quality Assessment-OSE	11(20.3)	12(17.3)
External quality Assessment-Panel testing	4 (7.4)	5 (7.2)
External quality Assessment-RBRC	9 (16.6)	20 (28.9)
<b>Infrastructure</b>		
Infrastructure-facilities	8 (14.8)	4 (5.7)
Infrastructure-HRD	12 (22.2)	6 (8.69)
Internal Quality Control	1 (1.84)	5(7.2)
SOP	1 (1.84)	0
Staining reagents / equipment	3 (5.55)	0
Training status	2 (3.7)	6(8.69)
Total recommendations	54	69

## Discussion

In India, RNTCP has rapidly expanded to cover one billion populations by the second quarter 2006.<sup>5</sup> Approximately, 0.8 million new sputum positive infectious TB cases are detected, annually. More than 11,000 designated microscopy centers offer the laboratory services.<sup>1</sup> To achieve the good quality and uniform results throughout the country, an inter-related quality assurance laboratory network is necessary.<sup>6,7</sup>

Situation analysis of laboratory services, globally, indicates limited implementation of revised guidelines of EQA/QA system for sputum smear microscopy. Difficulties were perceived in conducting and sustaining the NRL supervisory activities.<sup>18</sup> Failures to maintain the quality in the sputum microscopy would result in loss of credibility, wastage of resources, inaccurate data and poor performance of the programme.<sup>8</sup> Supervisory visits provide opportunity for on-the-spot corrections of identified shortcomings and ultimately would lead to improvements in case finding.<sup>9,10</sup> Systematic use of a standardized check-list during supervisory visits proved useful in improving the performance of the laboratories in TB diagnosis in Uganda and Ghana.<sup>10,11</sup>

Eight out of ten IRLs evaluated, Karnataka, Bihar, Jammu & Kashmir (Srinagar), Madhya Pradesh, Rajasthan, Orissa, Maharashtra and West Bengal were established one to two decades before the inception of RNTCP. NRL-OSEs strengthened these labs for the programme. After the first NRL-OSE visits, IRLs at Jharkhand and Pondicherry were established, within the existing TB sanatoria/hospital. Recognizing the importance of EQA, bigger states have added more personnel for conducting the district OSE visits. RNTCP phase II has strengthened this by additional recruitment of microbiologists.

Ready-made commercial stains were not of specified potency & concentration and affected

the quality in three states. While in one state (Jammu & Kashmir), all the three staining reagents –carbol fuchsin, sulphuric acid and methylene blue were from ready-made source, in other two states (Jharkhand and Orissa) only Carbol-fuchsin reagent was prepared, and other two were supplied ready-made. Effective annual maintenance contracts for preventive maintenance and repair of binocular microscopes were absent in some states.

Limited errors were detected in the Panel testing of IRL staff, indicating proficiency of IRL staff for sputum microscopy. Martinez et al., reported that technicians whose work was routinely rechecked had a higher proficiency than those whose work was not rechecked.<sup>12</sup> Evaluation of technical quality of smear microscopy in Argentine tuberculosis laboratory network emphasized the need for proper trainings and decentralized supervision activities.<sup>13</sup> Likewise, the results of panel testing at eight state-level laboratories in India emphasized periodic supervisory visits between national reference labs and state level labs, which in turn, supervise the peripheral labs.<sup>6</sup>

Prominent NRL-recommendations (repeated in more than one state) for district visits (table 10) focused on internal quality control, staining reagents/equipment and RBRC. Recommendations covered- usage and documentation of the control slides for quality checking of stains; potency correction for stains; discontinuing ready-made commercial reagents; providing weighing balances; replacement of cedar-wood oil with liquid paraffin; and discontinuation of Xylene for microscopy. To avoid 'rapid fading' of Ziehl-Neelsen stained smears which might lead to false negatives and grading errors during re-examination, a separate area for the RBRC and proper storage and transportation of rechecking slides formed a major recommendation.<sup>14,15</sup>

**Table 10 : Most often repeated OSE recommendations in ten states, year 2005**

---

**External quality Assessment-OSE**

1. Annual tour plan for conducting IRL OSE of District TB centers should be prepared, intimated to DTOs and adhered.
2. DTCs to submit action-taken-reports for IRL-OSE recommendations, within one month of OSE
3. *Prioritizing IRL-OSE visits*: OSE of the districts should be carried out giving priority to DTCs with High false results in RBRC; records and appropriateness of corrective actions initiated by DTOs in rectifying the errors

**External quality Assessment-Panel testing**

4. Equipments (Vortex-mixer, Bio-safety cabinet and Incubator) and chemicals (N-acetyl L-cysteine, Sodium Citrate etc.,) required for the panel slide preparations needs to be procured.

**External quality Assessment-RBRC**

5. STDC to review and rectify the annual LQAS sample size of DMCs of the state for RBRC, as per the recent EQA document.
6. Blinding procedure for RBRC should not be compromised. Coding & blinding register and Rechecking roster for controllers to be maintained under strict supervision of the DTOs.
7. RBRC errors need to be reported to STDC, monthly. STDC to monitor and report the DMCs with RBRC errors, with reasons for errors, in the state to NRLs.
8. Causes for High false errors in DMCs for RBRC to be investigated by the DTOs by making on-site visits. Corrective measures to be initiated without delay.

**Infrastructure-facilities**

9. STDCs should not function as DMC. Patient care management and Clinical activities performed by the STDC staff to be discontinued. Staff and lab of STDC need to be segregation from DMC laboratory
10. Mobility of the IRL-EQA OSE teams for districts visits to be ensured
11. Reorganization of existing STDC laboratory facilities or designating/identifying the existing public health laboratory facilities for IRL functions

**Infrastructure-HRD**

12. Vacant posts of Bacteriologist/EQA-officer and posts of LT at STDC to be filled.
13. Head of IRL/Director to be posted as full-time officer for supervision of IRL-EQA responsibilities.
14. Trained Microbiologist to be posted, full time, in IRL and not to be deputed in peripheral labs

**Staining reagents / equipment**

15. Weighing balances (preferably electronic) to be provided for reagent preparation at STDC
  16. Staining chemicals with known potency to be procurement at STDC. Potency correction factor for purity to be included while preparing the staining reagents.
-

Phenol (5% solution) would be more stable and effective as disinfectant for TB bacilli at high humidity conditions instead of commercial bleach (5-10%). Use of phenol formed the major recommendation for the infectious bio-waste disinfection. RNTCP has implemented the bio-waste management guidelines in 2005 and infectious waste management by burying in disposal-pits was advocated.<sup>16</sup> RNTCP revised LT modules training included these aspects.<sup>17</sup>

NRL-OSE recommendations focused on planning and prioritization of the OSE visits; obtaining action-taken-reports to assess implementation of IRL-recommendations; procurement of equipments for preparation of panel testing smears; verification of LQAS sample size for RBRC; completeness of blinding for re-examination of slides; taking corrective measures for DMCs with high false errors. Need-based reorganization of labs, discontinuing clinical activities and improving mobility of the OSE teams, in the field, were recommended.

NRL feedback mechanism to allow staff at peripheral levels to identify problems and areas where improvements are needed is lacking in many countries. Without a mechanism to follow up on recommendations formulated during an initial supervisory visit, subsequent visits become less efficient and operate as separate entities.<sup>18</sup> Our On-site supervisory visits to ten IRLs provided complete recommendations by the end of 3-4 day visit. A follow-up assessment on the extent of implementation of the recommendations was also carried out.

As a result of NRL OSE recommendations, the capacity of IRL was strengthened for staff and facilities. Errors that remained un-corrected by states pertained mainly to the improvements suggested for the district level infrastructure and RBRC activities.

Significantly, overall improvement for laboratory check-list items between two annual OSEs was 66.8%. No false error in some districts was mainly due to failure to completely blind RBRC slides for identity of LTs results. Incorrect interpretations of discrepant results lead to misclassification of errors. Blinding and re-examination of routine slides has been emphasized by a number of authors.<sup>19,20,21,22</sup>

The recommendations of the first year OSE were predominantly aimed at strengthening IRL capacity for EQA. Second year NRL recommendations laid emphasis on supervision of EQA-OSE and RBRC activities at peripheral labs, which were much desired. Recommendations were situation driven and were step-wise for successful EQA implementation, while enhancing the operational and technical ability of IRL.

### **Conclusions :**

To achieve the good quality and uniform results in TB sputum microscopy laboratories throughout India, an inter-related quality assurance laboratory network was established involving NRL, IRLs and peripheral labs. NRL conducted detailed On-site evaluations of IRLs, which act as supervisory link between NRLs and peripheral labs. As results of OSE visits, capacity of IRLs was strengthened for staff and facilities for EQA functions. Proficiency of IRL staff was tested for sputum microscopy. NRL-OSE recommendations for IRLs focused on planning and prioritization of the OSE visits, assessment of implementation of IRL-recommendations, RBRC procedure and taking corrective measures for DMCs with high false errors. Feed-back mechanism was developed for implementing recommendations between NRL and IRLs. Training to the lab personnel in preventive maintenance and minor repairs of binocular microscopes was required. District level activities for OSE and RBRC needed closer supervision.

IRL capacity for EQA was strengthening in the first year OSE. NRL recommendations in the second OSE, shifted towards improving EQA activities (RBRC) at peripheral labs, which was much desired.

**Acknowledgements :** We extend thanks to all the STOs, STDC Directors, STDC-microbiologists, WHO-RNTCP Medical consultants, and DTOs of ten states allotted to NTI- Bihar, J&K, Jharkhand, Pondicherry, Madhya Pradesh, Maharashtra, Karnataka, Rajasthan, Orissa and West Bengal- in India for extending their cooperation during the onsite evaluation and for periodic IRL-NRL reporting. The invaluable support provided by Dr. L S Chauhan, DDG

(TB), is gratefully acknowledged. We would like to thank Dr. Fraser wares, WHO-SEARO, Dr. S Sahu, WHO-India and Dr. Yamuna Mundade, WHO-RNTCP Medical consultant at Central TB division, New Delhi. We would like to thank all the LTs and STLS of the states allotted to NTI for their desire & dedication to improve their skills. We benefited from their extensive experience & knowledge of field conditions. Grateful thanks are extended to Dr. V K Chadha, Sr Epidemiologist, NTI, Bangalore for advice during the preparation of this manuscript. This work was supported with the RNTCP funds from Central TB division, Directorate General of Health Services, Min. of Health and Family welfare, New Delhi.

**Annexure 1- checklist**

**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	<b>Infrastructure:</b> 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	<b>Standard Operating Procedure:</b> 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	<b>Adequate stock and supply of:</b> Slides	Y / N	
10	Lens Tissue	Y / N	
11	Filter paper	Y / N	
12	Spirit lamp or Bunsen burner	Y / N	



Sl. No	Item	Adequate / Acceptable *	Problems Identified
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	
	<b>Staining reagents / equipment :</b>		<b>Within expiry date Y / N</b>
18	Carbol fuchsin	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	<b>Binocular Microscopes</b>	Y / N	
25	<b>Disposal of infected material :</b> Waste containers with lid	Y / N	
26	Waste disposal by Autoclave/disinfection/buried	Y / N	
27	General order/cleanliness	Y / N	
28	<b>Safety Practices</b>	Y / N	
29	<b>Training status:</b> 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	
31	<b>Internal Quality Control :</b> Control smears are used for each new batch of stain	Y / N	
32	<b>External quality control:</b> 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	

\* For Standards for reagents, refer to annexure-H of RNTCP laboratory network guidelines for quality assurance of smear microscopy for diagnosing tuberculosis<sup>3</sup>

**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 :*

iv) *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

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

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

## REFERENCES

1. RNTCP Annual report Central TB division, DGHS, Min of Health and Family Welfare, New Delhi. 2006. [www.tbcindia.org](http://www.tbcindia.org)
2. External quality assessment for AFB smear microscopy. PHL, CDC, IUALTD, KNCV, RIT, and WHO, Washington DC: Association of Public Health Laboratories, 2002.
3. RNTCP laboratory network guidelines for quality assurance of smear microscopy for diagnosing tuberculosis. Central TB division, DGHS, Min of Health and Family Welfare, New Delhi. 2005. [www.tbcindia.org](http://www.tbcindia.org)
4. Ajay Kumar T, Shyni San, Shilpa Shiju, Balasangameshwara VH, Kumar P. External quality assessment system for sputum smear microscopy: a short review of operational and technical implementation aspects in India. NTI bulletin 2005; 41/1&2, <http://ntiindia.kar.nic.in>
5. RNTCP performance report, India Second quarter 2006. Central TB division, DGHS, Min of Health and Family welfare, New Delhi. 2006. [www.tbcindia.org](http://www.tbcindia.org)
6. Paramasivan CN, Venkataraman P, Vasanthan JS, Rahman F, Narayanan PR. Quality Assurance studies in eight state tuberculosis laboratories in India. Int J Tuberc Lung Dis 2003; 6; 522-527.
7. Singh AA, Chauhan LS, State TB Training and Demonstration Centers: The Role in the revised National TB Control Programme. In: 191-194. [www.tbcindia.org](http://www.tbcindia.org)
8. Agarwal SP, Mundade Y, Chauhan LS. Quality assurance of sputum microscopy under the RNTCP. In: Agarwal SP, Chauhan LS, eds. Tuberculosis control in India. DGHS, Min of Health and Family welfare, India. New Delhi. Elsevier, 2005: pp 155-164. [www.tbcindia.org](http://www.tbcindia.org)
9. Hawken MP, Muhindi DW, Chakaya JM, Bhatt SM, Ng'ang'a LW, Porter JD. Under-diagnosis of smear-positive pulmonary tuberculosis in Nairobi, Kenya. Int J Tuberc Lung Dis.2001;5: 360-363
10. Aziz M, Bretzel G. Use of a standardised checklist to assess peripheral sputum smear microscopy laboratories for tuberculosis diagnosis in Uganda. Int J Tuberc Lung Dis 2002; 6; 340-349.
11. Addo KK, Owusu-Darko K, Dan-Dzide M, Yeboah-Manu D, Ablordey A, Caulley P, Minamikawa M, Bonsu F, Lienhardt C, Akpedonu P, Ofori-Adjei D. Situation Anaysis of TB Microscopy centers in Ghana. Int J Tuberc Lung Dis 2006; 10; 870-875.
12. Martinex G, Balandrano-Campos S, Ridderhof J, et al. Implementation of proficiency testing in conjunction with a rechecking system for external quality assurance in tuberculosis laboratories in Mexico. Int. J Tuberc Lung Dis 2003; 7: 516-521
13. Kuszniierz GF, Latini OA, Sequeira MD. Quality Assessment of smear microscopy for acid-fast bacilli in the Argentine tuberculosis laboratory network, 1983-2001. Int J Tuberc Lung Dis 2004; 8; 1234-1241.
14. Van Deun A. More on periphery to center quality control of sputum smear microscopy and 'rapid fading' of Ziehl-Neelsen staining.[In reply]. Int J Tuberc Lung Dis 2001; 5: 388-389.
15. De Kantor I, Laszlo A, Vazquez L, Reniero A, Latini O, Urbanczik R. Periphery to center quality control of sputum smear microscopy and 'rapid fading' of Ziehl-Neelsen staining [In reply]. Int J Tuberc Lung Dis 2001; 4: 887-888.

16. RNTCP-Environmental & bio-waste management plan for RNTCP II. Central TB division, DGHS, Min of Health and family welfare. New Dehli. 2005. [www.tbcindia.org](http://www.tbcindia.org)
17. Revised National Tuberculosis Control Programme. Module for Laboratory Technicians, Central TB division., Directorate General of Health Services, Min of Health and Family welfare, New Delhi, India. 2005. [www.tbcindia.org](http://www.tbcindia.org)
18. World Health Organisation. Strategic Approach for the Strengthening of Laboratory Services for Tuberculosis Control, 2006-2009. World health Organisation, Geneva; 2005. pp 3. [www.who.in](http://www.who.in)
19. Nguyen TN, Wells CD, Binkin NJ, Becerra JE, Pham DL, Nguyen VC. Quality control of smear microscopy for acid-fast bacilli: the case of blinded rechecking. *Int J Tuberc Lung Dis* 1999; 3: 55-61.
20. Martinez A, Balandrano S, Parissi A, Zuniga A, Sanchez M, Ridderhof J, Lipman H B, Madison B. Evaluation of new external quality assessment guidelines involving random blinded Rechecking of acid-fast bacilli smears in pilot project setting in Mexico. *Int J Tuberc Lung Dis* 2005; 9:301-305.
21. Selvakumar N, Prabhakaran E, Murthy BN, Sivagamasundari S, Vasanthan S, Govindaraju R, Perumal M, Wares F, Chauhan LS, Santha T, Narayanan PR. Application of lot sampling of sputum AFB smears for the assessment of microscopy centers. *Int J Tuberc Lung Dis* 2005; 9:306-309.
22. Van Deun A and Portaels F. Limitations and requirements for quality control of sputum smear microscopy for acid-fast bacilli. *Int J Tuberc Lung Dis* 1998; 2:756-765.