

**AWARENESS OF 'EXTERNAL QUALITY ASSESSMENT' NETWORK FOR AFB SPUTUM SMEAR MICROSCOPY & DRUG SENSITIVITY TESTING FOR *M.tuberculosis* AMONG POST-GRADUATE MEDICAL STUDENTS**

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**ABSTRACT**

Laboratory Diagnosis of Acid-fast bacilli by Sputum Smear Microscopy is an important component of Revised National Tuberculosis control Program (RNTCP). An effective External Quality Assessment (EQA) network would install confidence in RNTCP diagnostic algorithm. RNTCP has implemented specific programs such as Public Private Mix (PPM) involving the participation of Private Medical practitioners, Medical colleges, Non-Governmental organizations, and corporate hospitals. To assess the awareness of EQA structure and functional implementation aspects and for Drug resistance surveillance, a written survey was conducted. Postgraduate medical students attending Indian Association of Medical Microbiologists (IAMM) IX annual meeting of Karnataka Chapter participated in this study. A methodology of pre-test, structured-lecture and discussion, followed by a post-test that incorporated all the aspects of revised EQA guidelines for smear microscopy and finer aspects of drug sensitivity testing (DST) was adopted. The average performance of participants regarding questions on 'Methodology for Sputum smear- ZN staining and result grading as per RNTCP' and questions on 'mycobacterial culture and Drug resistance surveillance' were 44.5% and 30% for pre-test, that changed to 88.2% and 70.9%, respectively in the post-test assessment. The

average performance for questions on 'Structural organization of EQA network' and questions on 'Functional organization of EQA network' between pre and post test was from 22.7% to 54.4%, and 36.6% to 69.9%, respectively. The methodology used, and the questionnaire developed for this purpose could be used as an instrument for large-scale studies of this nature before RNTCP makes imperative policy decisions regarding EQA network in the private sector in India.

**INTRODUCTION**

Laboratory diagnosis of Acid-fast bacilli by Sputum Smear Microscopy is an important component of Revised National Tuberculosis Control programme<sup>(1)</sup>. Good quality Laboratory results are essential for proper initial categorization of the patients, decision to start the continuation phase, and to declare the outcome of treatment as 'cured' <sup>(2)</sup>. False results in diagnosis either lead to unnecessary treatment of the patient with potentially toxic drugs and puts precious resources of the programme to the drain, increasing the health care costs (false positive results); or deprive the potentially infectious TB patients from the benefit of treatment and cure (false negative results). Errors in reading follow-up of treatment smears can result in patients being placed on prolonged treatment or re-treatment, or in treatment discontinued prematurely<sup>(3)</sup>.

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Establishment and strengthening of good quality assured laboratory network structure within the administrative framework of RNTCP is a recognized and much emphasized priority <sup>(4, 5)</sup>. Quality assurance system comprises of internal 'bench-top' correct techniques and validated procedures (internal quality control-IQC) as well as external assessment methods (External quality assessment-EQA). An effective EQA programme will install confidence in RNTCP diagnostic algorithm and reduces the costs of running the control programme<sup>(6)</sup>. Anti-tuberculosis (TB) drug resistance is a major public health problem that threatens the success of DOTS. India has initiated measures to account for Multi Drug Resistant-Tuberculosis (MDR-TB) cases in the country by state-wise surveys for drug resistance (Drug resistance surveillance-DRS)<sup>(7,8)</sup>. DRS studies would also act as indicator for success rate of DOTS. An effective EQA network in all sectors is an essential step for DRS<sup>(9)</sup>.

The importance of participation of Private Medical practitioners, Medical colleges<sup>(10,11,12)</sup>, Non-Governmental Organizations and corporate hospitals at peripheral levels of DOTS<sup>(13)</sup> has been acknowledged and RNTCP network has implemented specific programs for Public Private Mix (PPM)<sup>(14)</sup> as a means to support the Govt. of India initiatives in achieving the RNTCP objectives. To assess the awareness of EQA structure &

functional implementation aspects and drug sensitivity testing for TB bacilli, a survey was carried out through pre and post-test evaluations among Post-graduate medical students pursuing their Masters programme. IX-IAMM annual meeting of Karnataka Chapter for the year 2004-05 held at Belgaum, Karnataka was selected for the survey, wherein a special Continuing Medical Education (CME) on Tuberculosis was also conducted.

## METHODOLOGY

The study was carried out with co-operation of IAMM at their IX annual meeting (Continuing Medical Education on tuberculosis was the theme) in the month of February 2005 at JN Medical College, Belgaum, Karnataka. Post-graduate Medical students pursuing MD course in Medical Microbiology in various colleges in the state of Karnataka attended the meeting.

A Test Questionnaire (annexure 1), of multiple-choice objective type, with sets of questions about EQA for smear microscopy and TB bacteriology was developed specifically for this purpose. The questions were arranged in following sets- Structural organization of EQA network, Functional organization of EQA network, Methodology for Sputum smear, ZN staining and result grading according to RNTCP, Myco-bacterial culture and Drug resistance surveillance (table 1).

**Table 1 : Description of questions set (annexure 1) :**

Question set	Question numbers (total number of questions)	Topic description
1.	1-5 (5)	Structural organization of EQA network
2.	6-13 (8)	Functional organization of EQA network
3.	14-18 (5)	Methodology for Sputum smear, ZN staining and result grading according to RNTCP
4.	19-25 (7)	Myco-bacterial culture and Drug resistance surveillance

Pre and post-test questionnaire consisted the same set of questions. The time given for the completion of the test was 20 min. Pre-test is followed by a structured-lecture presentation on EQA network in India, and demonstration on primary culture of mycobacterium, DST on solid Lowenstein Jensen medium- the method adopted for resistance surveillance<sup>(15)</sup>. A post-test was conducted after a thorough discussion. In addition, participants were also assessed in Proficiency in sputum smear staining and grading of 'Panel test smears' (results not given).

## RESULTS

The average performance of 22 participants during the Pre-test was 21.2%, 36.3%, 45.5% and 30%, respectively for the four question-sets (fig 1). The individual performance ranged from 9% to 60.6%.

The average post-test performance of the participants was 51%, 69.8%, 88.2%, and 71% respectively. The individual performance ranged from 30.3% to 90.9% (fig 1).

The average improvement between pre and post-tests for the question-sets was 29.8%, 33.5%, 42.7%, and 41%, respectively. While the overall average improvement was 35%, the individual improvement ranged between 9.0%-63.6% (fig 2).

The results for Acid-fast Bacilli counting and grading of Panel test smears, was without any major errors (data not shown).

## DISCUSSION

The awareness of participants pertaining to question set-3 (methodology for Sputum smear preparation, ZN staining and grading according to RNTCP) and question set 4 (Myco-bacterial culture and Drug resistance surveillance) were 44.5% and 30% for pre-test that changed to 88.2% and 70.9%, respectively in the post-test assessment. The awareness improvement for question set 1

(Structural organization of EQA network) and set 2 (Functional organization of EQA network) between pre and post test was from 22.7% to 54.4%, and 36.6% to 69.9%, respectively.

Although the overall awareness for RNTCP methodology for Sputum smear preparation-ZN staining-result grading was high, sufficient awareness on recent guidelines on EQA structural and functional network is lacking, indicating that Govt. of India efforts to implement the EQA for sputum microscopy have not yet fully reached the medical college level. At present, medical syllabus for the PG students does not include information on EQA network and recent advances in India for the DRS study.

Private Practitioners play a major role in TB care<sup>(10)</sup>. They contribute to DOTS expansion by working with the RNTCP and adhering to the national guidelines in offering DOTS care. The involvement of private medical practitioners/ corporate, specialty hospitals in EQA network helps in strengthening sputum microscopy laboratories, especially in their 'referral for diagnosis' activities<sup>(12)</sup>. Faculty at the microbiology departments of medical colleges has an important role in training students in the principles and rationale of EQA for smear microscopy and drug sensitivity testing<sup>(3)</sup> for TB bacilli among the medical and paramedical professionals.

The aim of EQA is on the identification and correction of laboratories where there may be serious systemic problems resulting in inaccurate results<sup>(3)</sup>. RNTCP has developed new EQA guidelines for implementation in the country incorporating the recent international guidelines<sup>(4, 6)</sup>. Both the availability and quality of AFB smear microscopy are dependent on participation of all affecting sectors and on good support, training and monitoring the testing performance of individual laboratories<sup>(17, 18)</sup>. As a policy, road map for TB control programmes in

India involves establishment of effectively functioning EQA network followed by DRS studies in the states, which would help in management of multidrug resistant (MDR) TB patients<sup>(7)</sup>.

The present study was carried out as an effort to assess the level of awareness on EQA functional network and DRS studies, in the medical college set-ups. The state of Karnataka has considerable number of public/private medical colleges (27 in number). As this study was carried out during an annual meeting of IAMM (Karnataka chapter), constraints such as, limited number of post-graduate students and skill levels, and representative nature of group are applicable. However, this meeting coincided with that of a special Continuing Medical Education (CME) on Tuberculosis allowing for practical demonstrations on ZN staining and grading according to EQA-RNTCP guidelines, and finer aspects of drug

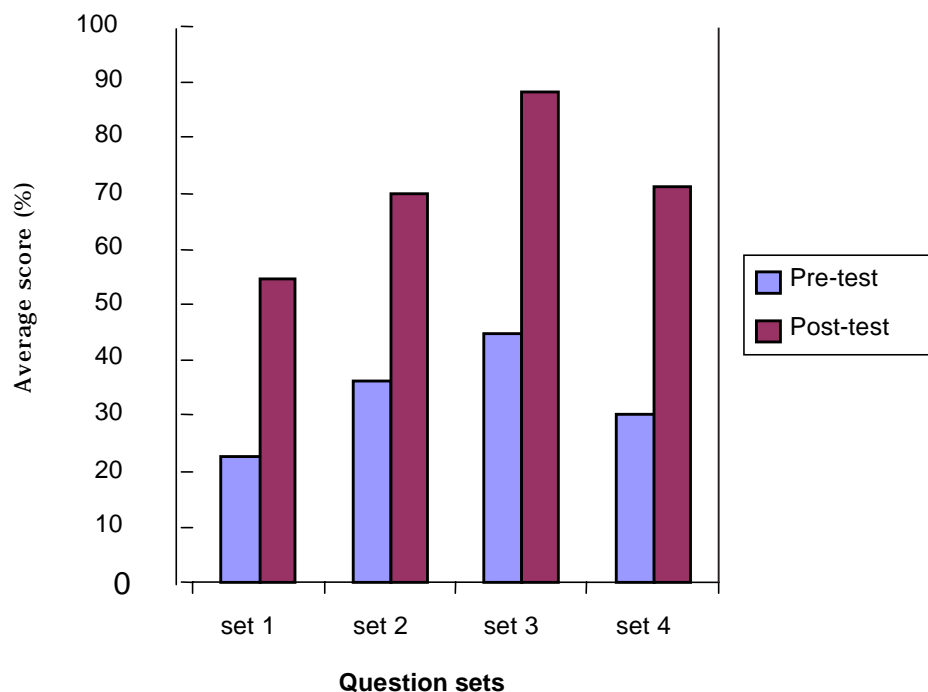
sensitivity testing (DST) and the method adopted for MDR-TB surveillance in India. Sensitization of medical college faculty in EQA, and their involvement will definitely help in achieving the quality assurance objectives of RNTCP.

The methodology used and questionnaire developed for this purpose could be adopted for large-scale studies of this nature before RNTCP makes imperative policy decisions regarding EQA network in the private sector.

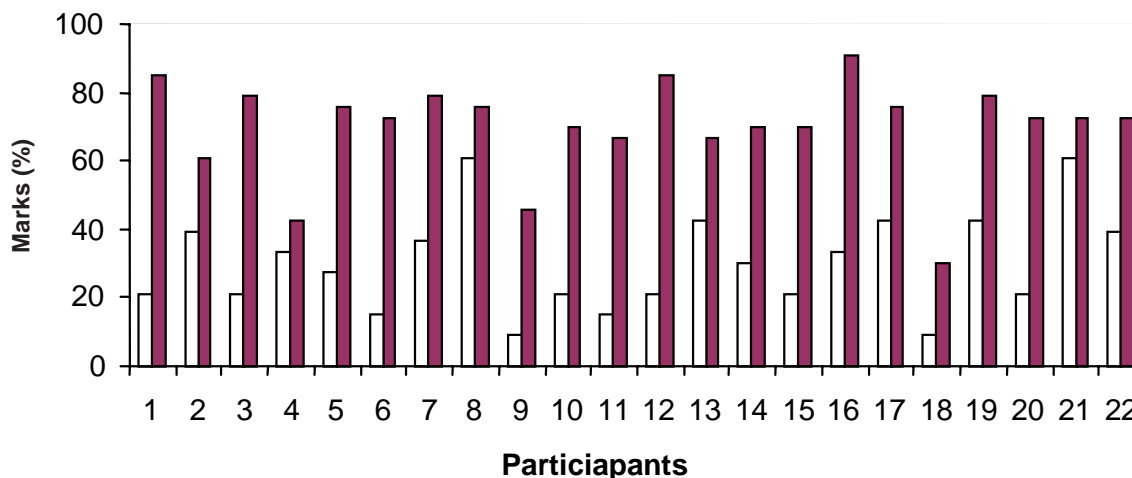
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**Fig 1 : Question set-wise performance of the participants**



**Fig 2 : Individual participants performance in Pre & Post-test assessment reflected in percentage.(Empty bars represent pretest and filled bars represent post test)**



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## ANNEXURE 1

### Test questionnaire for EQA for assessment of EQA awareness among post-graduate medical students during annual IAMM meeting

Name :

College :

**Circle correct answers; One question may have more than one correct answer.**

1. External quality assessment of sputum smear microscopy in tuberculosis has the following components
  - (a) culture by petroff's method
  - (b) on-site evaluation
  - (c) cold staining
  - (d) panel testing
  - (e) transport of specimens in cetyl pyridinium cholroide
  - (f) random blinded cross-checking of AFB slides
2. The role of EQA in patient care-management is
  - (a) it allows changes in patient diagnosis and treatment
  - (b) it should not be applied to patient care, at individual level
  - (c) retrospective analysis of errors, possible reasons leading corrective action to minimize the error from occurring in future.
  - (d) Prospective analysis of errors
3. List the components of EQA of peripheral labs, in the order of importance
  - (a)
  - (b)
  - (c)
4. The number and names of different levels in EQA are
  - (a) one, medical college
  - (b) two, Government and private
  - (c) three, national, intermediate and peripheral
  - (d) four, WHO, country, state and PHC
5. One of the important components of EQA of smear microscopy, without which one meaningful assessment can be made is
  - (a) ZN staining of three specimens for diagnosis
  - (b) On-site evaluation to evaluate the infrastructure, logistics, procedures, safety etc.,
  - (c) Panel testing to know the proficiency of peripheral lab workers
  - (d) Fluorescent microscopy for rapid detection of cases
6. One of the following is true for EQA of smear microscopy
  - (a) smear microscopy followed by culture is a prerequisite for diagnosis
  - (b) use of unstained manufactured panel slides during onsite evaluation is a preferred method for higher levels.
  - (c) Smear microscopy cannot diagnose more infectious forms of TB
7. In order to assess the quality of smear microscopy at peripheral centers under EQA
  - (a) examine all positives and 10% negative slides
  - (b) examine a maximum sample of all slides once a year, after using a blinded procedure
  - (c) examine a minimum number of statistically valid sample of all slides every month, using a blinding procedure
  - (d) examine 10% of all positive and 100% of negative slides.
8. The Sensitivity, specificity, acceptance value and confidence level for EQA under RNTCP, the following is true

- (a) 80%, 100%, 0 and 95%
  - (b) 100%, 80%, 0 and 99%
  - (c) 90%, 90%, 1 and 95%
  - (d) 95%, 80%, 3 and 99%
9. The internationally recommended method of statistical sampling for EQA of smear microscopy of TB is
- (a) unblended simple random sampling
  - (b) blinded population proportion cluster sampling
  - (c) blinded lot quality assurance sampling
  - (d) blinded 100% sampling
10. EQA aims at ;
- (a) Identification of errors, possible causes and actions needed to rectify the errors
  - (b) Identification of persons making any errors
  - (c) Identification of system responsible for errors
11. The recommended method of statistical sampling for EQA for smear microscopy under RNTCP
- (a) based on total slide volume anticipated for the next year, select maximum sample size following simple random sampling
  - (b) based on annual negative slide volume and the slide positivity rate to select the maximum monthly sample of slides method. LQAS.
  - (c) based on annual negative slide volume and the slide positivity rate to select the maximum monthly sample of slides method. cluster sampling.
12. The quality of smear microscopy is expected to be bad for any center performing
- (a) 500 to 1000 slides examinations/year by a single technician.
  - (b) Slide examination of more than 40 for a working by a single technician
  - (c) More than 500 slide examinations per year per technician
  - (d) Appox 10,000 slides/year by two technician
13. The positive results of Laboratory Technician is labeled as 'discrepant', if his results are;
- (a) one grade lower than controller's
  - (b) one grade higher than controller's
  - (c) two grade higher or lower than controller's
14. The concentration of stains used in smear microscopy under RNTCP are
- (a) 10% carbol fuchsin, 20% Sulphuric acid and 1% Methylene blue
  - (b) 1% carbol fuchsin, 25% Sulphuric acid and 0.1% Methylene blue
  - (c) 1% carbol fuchsin, 10% Sulphuric acid and 25% Methylene blue
  - (d) None of the above
15. The good quality sputum smear microscopy has the following characteristics under RNTCP
- (a) size of 1x2 cm(2), fairly thin
  - (b) size of 2x3 cm(2), not too thin, not too thick- prepared on any used slide
  - (c) size of 2x3 cm(2), not too thin, not too thick prepared on a greese-free new slide.
  - (d) size of 1x3 cm(2), not too thin, not too thick prepared on a greese-free new slide.
16. Grading of sputum smear microscopy for AFB under RNTCP, one of the following is true:
- (a) minimum of 10 fields per negative slides, 1+, 2+, 3+ and a scanty
  - (b) minimum of 50 fields per negative slides ,20 fields for 1+, 100 fields For 3+ and 2+ and scanty
  - (c) minimum of 100 fields per negative slides and 1+ ,20 fields for 3+, 50 fields for 2+ and 200 fields for scanty.



- (d) minimum of 100 fields per negative slides, 1+, 2+, 3+ and a scanty.
17. A stained smear stored for more than one month is likely
- to have same grade positivity
  - does not fade
  - Likely to have faded and required restaining
  - none of the above
18. The number of slides for diagnosis and follow-up of the TB patients are
- one each respectively
  - two each respectively
  - three each respectively
  - two and three each respectively
  - three and two each respectively
19. Indications for culture and sensitivity for TB patients are
- all newly diagnosed smear positive, smear negative and extra-pulmonary cases
  - all patients failed on retreatment and mainly seriously ill extra-pulmonary cases
  - for drug resistance surveillance
  - all TB patients
20. For large states in India the recommended sampling procedure for DRS is
- simple random sampling
  - Population proportion cluster sampling
  - lot quality assurance sampling
  - 100% sampling
21. The recommended Drug sensitivity procedure for DRS is
- resistance ratio
  - minimum inhibitory concentration
  - economic variant of proportion method
  - Bac-tec
  - All of the above
  - None of the above
22. The proportion of drug resistant population required for label to the isolate as "resistant" is
- more than or equal to 10%
  - more than or equal to 1%
  - less than or equal to 10%
  - less than or equal to 1%
23. The concentration of drug used in proportion method for Isoniazid, Ethambutol, streptomycin and rifampicin drugs ( in ug/ml) are
- 2, 0.2, 40, 4
  - 0.2, 2, 4, 40
  - 40, 4, 2, 20
  - 4, 40, 20, 2
24. One of the following test is sufficient to identify mycobacterium tuberculosis
- no growth in LJ medium (500ug/ml) of p-nitrobenzoic acid
  - nicacin negative
  - nitrate reduction test negative
  - catalase-peroxidase test at 68c negative
25. The dilutions used to set up a DST for proportion method are
- neat,  $10^{-2}$ , and  $10^{-4}$
  - neat,  $10^{-3}$ , and  $10^{-5}$
  - neat,  $10^{-1}$ , and  $10^{-3}$
  - neat only.