

Special Article

Role of laboratory in RNTCP

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Tuberculosis (TB) remains a major public health problem in many parts of the world. Sputum smear microscopy is the mainstay of diagnosis of TB. RNTCP follows the international guidelines which recommend the establishment of microscopy centre for every 100,000 population. All patients with a cough of three weeks or more should undergo 3 sputum diagnostic examinations for acid-fast bacilli (AFB). A separate TB laboratory register is maintained in each microscopy centre. Disposal of laboratory waste should be destroyed. One slide from each patient is sent to the District TB Centre (DTC) for external quality assurance (EQA). The activities of the microscopy centres in a TB unit are supervised by a Senior TB Laboratory Supervisor (STLS). The STLS visits every microscopy centre at least once a month. Sputum samples from patients who fail RNTCP treatment are sent to the nearest reference laboratory for culture of *M tuberculosis* and drug susceptibility testing; however there is no need to send patient samples routinely for culture.

Key words : Tuberculosis, sputum smear microscopy, RNTCP guidelines.

Tuberculosis (TB) remains a major public health problem in many parts of the world. Whilst several hi-tech diagnostic procedures for TB are under development, the mainstay of diagnosis of pulmonary TB is still sputum smear microscopy, which despite being 120 years old, is relatively simple, inexpensive, highly specific and rapid.

Microscopy Centre :

The RNTCP follows the international guidelines which recommend the establishment of a microscopy centre (MC) for every 100,000 population. A trained laboratory technician (LT) is responsible for all activities in an MC. All patients with a cough of three weeks or more duration should undergo 3 sputum diagnostic examinations for acid-fast bacilli (AFB). Sputum samples should be collected in an open place or in a highly ventilated room after suitable instructions have been given to the patient. Smears are made, stained by the Ziehl-Neelsen (ZN) method, and results graded as per RNTCP guidelines (Table 1).

Observation	Result	Grade	Minimum number Of fields to be examined
More than 10 AFB per OIF in at least 20 fields	Positive	3+	20
1-10 AFB per OIF	Positive	2+	50
10-99 AFB per 100 OIF	Positive	1+	100
1-9 AFB per 100 OIF	Scanty	Record exact number seen	200
No AFB per 100 OIF	Negative	—	100
OIF - Oil immersion field			

Category of treatment	Smear result at end of intensive phase	Month of examinations
Cat I	Negative	At the end of months 2, 4 and 6
	Positive	At the end of months 3, 5 and 7
Cat II	Negative	At the end of months 3, 5 and 8
	Positive	At the end of months 4, 6 and 9
Cat III	Negative	At the end of months 2 and 6

During the course of treatment, 2 sputum samples are examined according to a fixed schedule to monitor the progress of treatment in the individual patient (Table 2).

Documentation :

A separate TB laboratory register is maintained in each MC. The details such as patient's name, complete address, age, sex, type of the disease, whether it is for diagnosis or treatment, are all entered in the register. The laboratory serial number, with the highest grade result among the 3 diagnostic samples and the 2 follow-up samples, is entered on the individual patient's treatment card. This information is also entered in the TB register by the Senior Treatment Supervisor during their monthly visits.

At the end of every month, an abstract is prepared containing the information in the TB laboratory register on the number of

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symptomatics examined, number of smear positive patients diagnosed, number of patients examined for follow-up, and number of patients found to be smear positive on follow-up examination. The sputum conversion rate at the end of intensive phase of treatment, calculated for a quarterly cohort of patients at the TB Unit (TU) level, reveals the effectiveness of the implementation of RNTCP. The cure rate calculated for quarterly cohorts of patients, indicates the performance of the RNTCP.

Disposal of Laboratory Waste :

All infective materials (eg. sputum cups, sticks and slides) are placed overnight in a vessel containing either 5% phenol or 5% freshly prepared bleach for decontamination prior to proper disposal. The positive slides should never be used again and should be destroyed and correctly disposed of at the District TB Centre (DTC).

Network of Microscopy Centres :

The activities of the MCs in a TU are supervised by a Senior TB Laboratory Supervisor (STLS). The STLS visits every MC at least once a month. They ensure an adequate supply of laboratory materials to the MC and the provision of uninterrupted smear microscopy services, in co-ordination with the Medical Officer for TB Control at the TU. During site visits, the STLS reviews all positive and 5 randomly selected negative slides. Any discrepancy in the result is recoded in the remarks column of the TB laboratory register, with concurrence from the respective LT. A supervision visit report is prepared and given to the Medical Officer of the Health Unit for their perusal. The District TB Officer convenes a meeting of all STLSs at the DTC once a month to discuss the managerial issues of the laboratories.

External Quality Assurance (EQA) of Sputum Smear Microscopy:

One slide (either A/B/C) from each patient is sent to the DTC for EQA. Twenty slides from each MC are then selected for re-checking by one of the STLSs. Discrepant smears are checked by a second STLS and this umpire reader's result is considered final. A two-way table comparing the results of the LT and STLS is prepared, performance evaluated, and LTs in need of re-training are identified. The QA table is sent as part of the quarterly report from each of the TU to the Central TB Division, with the performance of smear microscopy in each TU, District, State and the whole country being assessed.

Panel Testing :

National Institutes (TB Research Centre and the National TB Institute) are sending 50 quality control (QC) slides to each of the 16 State TB Training and Demonstration Centres (STDCs) once every 6 months to be read by the LTs in the STDC. The results are sent back to the respective National Institute within a month. Similarly once every 6 months, 25 QC slides are sent from the STDCs to each DTC in the respective state for reading by the all STLSs in that district, with the results sent back to the STDC within a month. The reading capability of the LTs and STLSs are assessed, and if indicated re-training is organised. At the TU level, once a month, 3 prefixed and unstained QC slides are given to the LTs by the STLSs for staining and grading to ensure the quality of staining reagents, correctness of staining procedure and grading. Any deficiency in the technical procedure is immediately corrected.

Training :

All STLSs in the State receive 15 days training in sputum AFB smear microscopy, either at an STDC or a National Institute. All LTs in a district are trained at the DTC. Every five years, all LTs and STLSs are given refresher training. In a number of states, the microbiology departments in Medical Colleges are also involved in the training of LTs and STLS.

Culture of Mycobacterium Tuberculosis :

Sputum samples from patients who fail RNTCP treatment are sent to the nearest reference laboratory for culture of *M tuberculosis* and drug susceptibility testing. There is no need however to routinely send patient samples for the culture of *M tuberculosis*.