

## **Contribution of National Tuberculosis Institute Laboratory to TB Control in India**

---

### **Tuberculosis scenario in India:**

1. India has the highest TB burden accounting to one fifth of the global burden.
2. RNTCP is the largest and the fastest expanding TB control programme in the world.
3. By, 2009 the programme had achieved the treatment success rate of 87% and case detection rate of 72% among new sputum positive (NSP) cases.
4. Quality assured sputum smear microscopy services are available through more than 12,500 sputum microscopy laboratories in the health system across the country.
5. The prevalence of Multi Drug Resistant TB (MDR-TB) is estimated to be less than 3% in new cases and 14-17% in retreatment cases.

### **NTI-Introduction and History**

The pioneering work undertaken by the Indian Council of Medical Research, New Delhi, “National Sample Survey of TB in India”, initiated the formulation of a strategy to control Tuberculosis in India. This laid the foundation for establishing ‘National Tuberculosis Institute’, Bangalore, under the Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi. NTI was formally inaugurated on 16<sup>th</sup> September 1960 by Late Pandit Jawaharlal Nehru, the first Prime Minister of India. The National survey was supported by international agencies such as World Health Organization (WHO) and United Nations International Children’s Emergency Fund (UNICEF). The concerted efforts of late Dr. P.V.Benjamin, then advisor on TB to the Government of India and Dr. H.T.Mahler of WHO, further gave a lasting impact on the TB control program in India. Due to their motivation and guidance, all TB workers of India joined hands to fight the TB menace in India which was one of the greatest challenges faced by the country during that period.

Robert Koch Memorial Block: Houses the main laboratory for Solid culture, Liquid culture, Molecular Biology, High Performance Liquid Chromatography (HPLC) and line Probe Assay (LPA), apart from the Board room and the Silver jubilee hall.

PV Benjamin Memorial Block: Houses the Library & Publication wing, Statistics and Monitoring sections, Training section and class rooms, Conference hall and the Computer section

Animal Model Research Unit: Includes BSL-III facilities for testing-Mycobacterial proteins (future vaccine candidates) and also houses the International Centre of Excellence in Laboratory Trainings(ICELT).

History of any institution encompasses the events, time, places, organizations and the people involved in it. In the growth process, NTI since its formation has been nurtured by the Government of Karnataka, World Health Organization (WHO) and the United Nations International Children’s Emergency Fund (UNICEF). During these fifty years, the staff of NTI

and WHO officers posted to NTI, worked diligently towards the revolutionary approach to control TB in the country. As we celebrate the Golden Jubilee of NTI this year, we look back to the genesis of this landmark in the nation's efforts towards TB control. NTI was established with the prime purpose of formulating a nationally applicable programme for TB control by training key TB personnel, officers and other workers. Apart from this, NTI was also monitoring the National Tuberculosis programme (NTP) and was assisting in the development of the laboratory infrastructure in the states.

The official website of the Institute <http://ntiindia.kar.nic.in> was launched on 15<sup>th</sup> August 2001.

## LABORATORY

### Introduction:

Clinicians, TB-control officials, public and private health officials need access to prompt and reliable TB laboratory services to eliminate TB. Delayed laboratory confirmation of TB leads to delays in initiation of therapy, potentially inappropriate therapy, and missed opportunities to prevent transmission. Although provision of laboratory services is a jurisdictional matter and can be organized at the local, state, or regional level, any successful effort to provide prompt, reliable laboratory services should involve assessment and understanding of the structure, performance, and cost of the network of laboratory service providers and users, development of a referral and information network to ensure reliable testing and prompt flow of specimens and information and use of quality-improvement principles to continually evaluate and improve the performance of the laboratory service network.

Rapid detection, species identification, and testing for drug resistance are necessary to control TB among patients and populations. To optimize diagnosis, flow of specimens and information should be efficient and well-coordinated which is well taken care of at NTI. Turnaround times, maintenance of Proficiency standards & capacity and capability assessment of the NTI laboratory is done regularly, to determine the status and capacity of services, to identify unmet needs, obstacles to obtaining laboratory services, and opportunities for improvement.

The facilities provided by the NTI laboratory include Sputum smear microscopy by both the conventional Ziehl Neelsen and Fluorescent staining methods. Besides supporting projects such as Drug Resistance Surveillance (DRS) and DOTS Plus at the state level, the laboratory also supports projects of Animal Model Research unit and the Disease Prevalence Surveys (DPS).

### History:

Considering the magnitude of the problem posed by Tuberculosis and as depicted by the ICMR survey of 1955-58, there was a felt need for formulating a program addressing important issues related to its control. The survey results showed that there were four sputum smear positive cases and sixteen radiologically positive cases of pulmonary TB per thousand population. This observation was found to be equally distributed in rural and urban areas. For this study the technical inputs in terms of manpower, machinery and equipment came from the WHO, assistance with vehicles and equipments was provided by UNICEF. These were of great value during the formative years of NTI. The institute became a centre for formulating a TB control Programme best suited to a country like India, where the TB problem was large and the resources were meager. The Institute undertook several Operational Research studies towards this end and evolved a nationally applicable TB Control Programme (NTP).

The Government of India reviewed the TB program in depth with collaboration of WHO in the year 1992 and documented all the known inadequacies and remedial measures of the program. This was followed by the birth of Revised National TB Control Programme (RNTCP).

From 1993 onwards, NTI started to conduct training courses on RNTCP- the Modular Trainings, where key TB personnel in different states of India were imparted training by the laboratory staff along with other senior members of the institute. In the year 1997 faculty from Central TB division including DDG along with other senior members came to NTI as facilitators to train the participants, this showed the dedication of the top most people linked to the program.

During the inception of the institute the laboratory set up was in the main building (now the Administrative Block) in a small section. The entire laboratory was later shifted from the main building to the new premises- 'The Robert Koch Block'. Now two complete wings on the ground floor and the first floor of the Robert Koch Block have been designated for the laboratory. During those early years the laboratory section undertook several research studies to come up with very high standard laboratory procedures. Finally in 1970, a very important document 'Bacteriological diagnosis of pulmonary TB- Sputum Microscopy' was published, then in 1974 Dr Naganathan published an article: 'Some guidelines for establishing a TB Culture laboratory', this gave a good insight on initiating the development of new TB laboratories

Now NTI has the nobility of being a premier training institute which imparts laboratory based training programmes in 'Culture and Drug Susceptibility Testing of *Mycobacterium tuberculosis*' and 'External Quality Assurance (EQA) of sputum smear microscopy' for all the State laboratory personnel in India.

#### Organizational structure

The Mycobacteriology laboratory at NTI is a strong component of the Institute. It is designated as one of the four National Reference Laboratories (NRL) in India for

undertaking the Quality Assurance of Sputum Smear Microscopy and Culture and Drug Susceptibility Testing (C&DST) in the TB laboratory network, under RNTCP. The laboratory conducts training programmes in EQA and C&DST for State laboratory personnel and participates in the accreditation of state level Intermediate Reference Laboratories (IRLs), Medical Colleges and other private laboratories for Culture and Drug Susceptibility Testing. It also provides technical support to SAARC TB centre, Kathmandu and a WHO collaborating centre.

### Training Activities

The training of in-service medical and paramedical personnel was one of the major activities of the laboratory section. This further constituted a team of trained manpower who used to impart training to the subordinate counter parts at sub district level. With time, the duration of training shortened from 6 months to few weeks based on technical and operation factors, without compromising with the quality of training in the growth process.

One of the first research protocols at NTI was a pilot protocol for training the newly recruited staff of NTI, twenty one BCG technicians and three senior health inspectors deputed from Mysore state (now known as Karnataka state). They were imparted training in field work supervision, processing of data, reading MMR films, processing sputum for smear microscopy, culture and other laboratory based tests. Two LTs of NTI were trained in all aspects of laboratory work like sputum smear microscopy, culture and drug sensitivity for *Mycobacterium tuberculosis*.

Gradually detailed work manuals were prepared for the training of District TB Officer (DTO), Treatment Organizer (TO), Lab Technician (LT), Statistician, X-ray Technician (XT) & various other bodies. The first edition of the manual was issued in 1962 after being approved by the concerned authorities.

### Research and Technical Development

The laboratory at NTI is the backbone of the Institute where in the past fifty years many new research findings in the field of TB diagnosis, control and related areas came up.

Many research Protocols were planned for various studies covering different aspects of TB control during the year 1961. A technical coordinating Committee (TCC) was constituted, headed by the Director with officers from each section of NTI as its members. A TCC room was allotted for this purpose and weekly meetings were held by the committee and draft protocols were elaborated, presented and discussed over for the approval of TCC. This activity enriched the protocols with different ideas and also the members became aware of the activities undertaken by different sections and the institute as a whole. TCC was thus a hard testing ground for all good ideas and the successful ones would take the shape of research projects or protocols that would further be taken up for field work. The TCC also reviewed the work in progress and after completion of the work by reviewing the outcome, before publication of the results.

In 1995, two booklets “What we should know about TB, its diagnosis, treatment and prevention” and “TB and HIV” were published. Next to this, in 1996, the chart “Guidelines for laboratory procedures in TB” was published. During this entire duration WHO continued to grant funds for publishing important documents and manuals and it also assisted in acquiring imported equipment for the laboratory.

To be proficient, the laboratory participates in the annual proficiency testing for Culture and DST with the Prince Leopold Institute of Tropical Medicine, the Supra-National Reference Laboratory (SNRL) located at Antwerp, Belgium. NTI is proficient in Drug Susceptibility Testing for both first and second line drugs of Tuberculosis apart from starting the new liquid culture and molecular techniques which assist in quicker diagnosis and further patient treatment. The present Director General-ICMR formally inaugurated the Second line DST facility at NTI on 16<sup>th</sup> September 2008. The in-house training for the newer techniques of *M. tuberculosis* diagnosis is in progress for the section staff and gradually all the laboratory staff will be trained as bench workers and facilitators for the new diagnostic techniques. With the new developments in the section the institute will have all the advanced facilities for training the manpower in new techniques and the rapid diagnosis of *Mycobacterium tuberculosis*.

Around 1998 NTI put into operation the Quality Assurance in Sputum Microscopy under RNTCP. In this modalities were developed and tested to send a set of hundred blinded slides to the STC's and DTC's once in six months for examination and finally being returned back. Results were analyzed and action taken accordingly. During the same time a manual on “Isolation, identification and Sensitivity testing of Mycobacterium TB, Ed.2” was also published from the section.

The Institute is also involved in building a spectral library of the chromatographic profiles of the Mycolic acids of Pathogenic Non-tuberculous Mycobacterium (NTM) using High Performance Liquid Chromatography (HPLC).

RNTCP has established a Nation-wide laboratory network, encompassing over 12,500 designated Sputum Microscopy Centers (DMCs), which are being supervised by Intermediate reference laboratories (IRL) at the state level, the National Reference Laboratories (NRL) and Central TB Division at the National level. The Quality Assurance programme consists of Internal Quality Control, External Quality Assessment and Quality Improvement. It involves classification of errors in sputum smear microscopy and assessment of laboratories based on Lot Quality Assurance Sampling (LQAS). The laboratory staff at NTI, facilitate in National and International trainings and monitor the External Quality Assurance (EQA) for the nine allotted states. In this the NRL Microbiologist and laboratory supervisor/technician visits each assigned state at least once a year for 2-3 days as a part of on-site evaluation under the RNTCP EQA protocol. Regular supervisory visits are undertaken by the NRL Microbiologists to the IRLs to provide technical support for establishing quality assured C&DST services. NRLs also undertake periodic proficiency testing of the IRLs as part of the accreditation process under RNTCP. Training is imparted to the state level programme managers in EQA to make them aware of

their roles and responsibilities. A separate training which focuses mainly on the technical aspects of EQA protocol is provided at NTI to the IRL Microbiologists and the laboratory technicians.

Once the laboratories are found proficient in performing Culture and DST as per the RNTCP guidelines, Culture and DST Laboratory Accreditation is the next step for Public and private laboratories. This provides accurate and reliable services for MDR-TB Diagnosis and follow-up of treatment. The accredited laboratory regularly and satisfactorily participates in the Proficiency Testing program being conducted by National Reference Laboratory (NRL) and their satisfactory performance is determined in terms of concordance of >90% for Isoniazid(H) & Rifampicin(R) and >80% for Streptomycin(S)& Ethambutol(E). NTI has accredited five IRLs Ajmer in Rajasthan, Nagpur in Maharashtra, Calcutta in West Bengal, Cuttack in Orissa and Ranchi in Jharkhand and one Government Medical College- S M S Medical College, Jaipur

Among the path breaking studies a study conducted in the initial phases (1965)<sup>1</sup> was on the operational factors influencing the relative utility of culture method for diagnosis of pulmonary TB. This study suggested that the additional case detection by culture depends on preliminary selection of cases. It also suggested that the interval of one to seven days between collection and inoculation did not adversely affect the yield of positive cultures. The rate of contamination was also not affected by the time interval only if collection was as per the guidelines.

In another study conducted in the section in 1987<sup>2</sup>, on the recovery of tubercle bacilli from urine of pulmonary tuberculosis patients was compared with the corresponding sputum isolates. It was observed that a significant number of these patients yielded urine isolates which differed from the corresponding sputum isolates in drug sensitivity profile, virulence for guinea pigs and phage type. This signified the different episodes of infection in the same patient.

An investigation was carried out in 1995<sup>3</sup> to assess the values of ELISA test in the serodiagnosis of pulmonary tuberculosis and the effectiveness of two ELISA test kits, using A60 and KP90 antigens respectively. Sputum culture was performed to confirm the diagnosis. Serum dilution of 1:100 was used and IgG antibodies to A60 and IgA antibodies to KP90 were looked for. A total of 317 patients were studied. The cut-off point (mean + SD) was taken from healthy controls. The Sensitivity, specificity and accuracy of KP90 ELISA were 49.7%, 84.0% and 61.5 % and those of A60 ELISA were 48.3 %, 92.0% and 71.3% respectively. It was considered that in high prevalence conditions, serological tests for tuberculosis based on antibody detection should be used and interpreted with extreme caution. The results should definitely score over sputum microscopy results in order to be widely applicable.

In the year 1995<sup>4</sup>, the virulence and sensitivity patterns of *M. tuberculosis* grown in an atmosphere of 10% CO<sub>2</sub> (A) and without 10% CO<sub>2</sub> (B), 219 isolates were examined. Of the total isolates, 98 (44.7 percent) were found sensitive to all drugs and the remaining 121 (55.3 percent) resistant to one or more drugs. Method A was not superior to method

B for the growth of drug resistant Mycobacteria, but for the growth of strains sensitive to all drugs, method A was superior to method B. For INH sensitive strains subjected to virulence study, method A was superior to method B and the percentages of low and high virulence were 73.8 and 26.2 for method A compared to 45.7 and 54.3 for method B respectively. Thus it was observed that the presence of 10% CO<sub>2</sub> enhances the growth of sensitive bacilli and facilitates the detection of low virulence isolates sensitive to INH.

In 1996<sup>5</sup> a study was published on the Assessment of Trisodium Phosphate (13% solution of TSP with Benzalkonium chloride) for storage and isolation of Mycobacteria in single step culture method. It was observed that TSP method was less lethal to Mycobacteria as well as had less contamination rates too. In smear positive specimens, culture positivity was found to be more as compared to Modified Petroffs method. The method was also advantageous as it did not require any sophisticated equipment nor technically skilled personnel.

A Comparative study between rapid colorimetric Mycobacterial isolation and Susceptibility testing method and Conventional method using LJ Medium was undertaken during 2000<sup>6</sup> and it was found that the Colorimetric method enables rapid detection and drug susceptibility testing, making it possible to get the results four weeks earlier as compared to the conventional LJ method. The major constraint of MB-BacT system was the cost of the equipment and the processing cost per specimen basis, this was very high when compared to the Conventional method using LJ Medium.

A pilot study on Drug Resistance Surveillance was undertaken by NTI in the year 2003<sup>7</sup> in the districts of Mayurbhanj (Orissa), Hoogly (W.Bengal) and Nagaon (Assam), after Central TB Division and Ministry of Health and Family welfare and Government of India reviewed the available data on Multi-Drug Resistance (MDR) in the Indian context. During the same time one more study was conducted on the Drug Resistance Surveillance in Mysore district. The objective was to measure the levels and pattern of resistance to anti-tuberculosis drugs among newly diagnosed sputum smear positive pulmonary cases in two districts. It was observed that the resistance levels to all the four primary drugs ranged from 0.4% to 3.9% in Mayurbhanj and 1.9% to 13.7% in Hoogli district. MDR was 0.7% (95% CI:0.0%-1.7%) and 3.0% (95% CI: 1%-5.1%) in Mayurbhanj and Hoogli districts respectively. These studies were undertaken to see the trend in Initial Drug Resistance in selected districts of India. These studies demonstrated that the levels of Isoniazid, Rifampicin and MDR was within the expected levels, when compared to other studies conducted in India as per the global DRS guidelines. These studies served as a baseline for conducting the DRS of Maharashtra state. Experimental research was an integral part of the laboratory during that time and subsequently a highly remarkable project was awarded to NTI for "Surveillance of Drug Resistance in Maharashtra". This project was an indicator for the program quality and it showed the proportion of patients with initial (2.7%) and acquired drug resistance (13.7%) in the state of Maharashtra.

The section continues to grow with the addition of International centre of excellence in training for LPA.

## Major Studies undertaken by the Bacteriology Section

- 1961 : A pilot investigation for the detection of isoniazid in urine by a simple impregnated filter paper method.
- 1962 : Technical assessment of a field culture inoculation technique carried out in a district laboratory and a comparison of spot and overnight specimen in mass case finding.
- 1962: Assessment of house to house BCG work as integrated with district tuberculosis programme, Anantpur.
- 1962: Bacteriological aspects of mass chemotherapy with thiacetazone as an adjuvant to isoniazid among tuberculosis patients in Tumkur town.
- 1962: Comparison of fresh liquid and freeze-dried BCG vaccines produced from Danish and Japanese strains in BCG vaccine laboratory, Madras.
- 1963 : Assessment of sputum examination carried out in selected microscopy centres of the Bangalore district programme over a 12 month period.
- 1964 : A comparison of cold staining with the conventional Ziehl-Neelsen method.
- 1967 : Variation in examination of positive sputum specimens by direct microscopy.
- 1968 : Cold staining method for differentiating *Mycobacterium tuberculosis* and other acid fast bacilli.
- 1972 : Stainability of tubercle bacilli with different brands of basic fuchsin.
- 1973 : Comparison of conventional ZN method of staining with and without Alcohol decolorisation and staining by Gabbets Methylene blue.
- 1975 : Evaluation of smears prepared in different ways.
- 1976 : Pilot study on some bacteriological aspects among patients attending, Lady Willington TB demonstration and Training Centre, Bangalore.
- 1977 : Preliminary study to investigate the influence of coconut water on the growth of tubercle bacilli.
- 1978 : Comparison of results of homogenization and decontamination of sputum specimens with different volumes of sodium hydroxide.



- 1979 : Some methods to improve case finding by microscopy in pulmonary tuberculosis.
- 1982 : Study to differentiate viable tubercle bacilli from dead ones in sputum smears by staining.
- 1983 : Virulence of *M.tuberculosis* isolated in Bangalore area.
- 1984 : Behavior of *M.tb* of low virulence on animal passage.
- 1985 : Recovery of tubercle bacilli from wine and potential contribution of virulence in haematogenous forms of tuberculosis.
- 1987 : Rate of Mycobacteria other than *M.tb* in immunization against tuberculosis.
- 1987 : Haematogenous dissemination of tubercle bacilli in guinea pigs and its predictive value for pathogenesis of human tuberculosis.
- 1987: Screening fresh isolates of *M.tb* homogeneity in virulence, morphology and pigmentation of colonies.
- 1987 : Serodiagnosis of pulmonary tuberculosis by kaolin agglutination technique.
- 1988: Use of trisodium phosphate and cetyl pyridinium chloride(CPC) as transport media for sputum specimens in isolation of *Mycobacterium tuberculosis*.
- 1988 : Serodiagnosis of extra pulmonary tuberculosis by ELISA and passive haemagglutination (PHA) tests.
- 1989 : Serodiagnosis of pulmonary tuberculosis by enzyme linked immunobrosant assay (ELISA) and passive haemagglutination tests.
- 1990: Effect of 10% carbon dioxide on the primary isolation of Mycobacteria.
- 1990: Biological characteristics of *M.tuberculosis* isolated from urine and corresponding sputum specimen in a series of patients with pulmonary tuberculosis.
- 1990: Sero-epidemiology of tuberculosis in children aged 0-14 years in areas around Bangalore.
- 1990: Evaluation of cold staining methods for acid fast bacilli using various easily available detergents and candle flame.
- 1990: Evaluation of culture methods using N-Acety-L-Cysteine and 2% sodium hydroxide and 7H10-agar based culture medium.

- 1991: Isolation of environmental mycobacteria from soil and water.
- 1997: Assessment of the trisodium phosphate-penicillin transport medium and single step method of culture.
- 1998: Surveillance of drug resistance in Mysore district.
- 2000: Prevalence of chest symptoms and utilization of health services in a peri urban community.
- 2001: Status of Pulmonary TB patients put on treatment under RNTCP from Bangalore Mahanagar Palike.
- 2001: Treatment outcome of smear positive pulmonary tuberculosis patients in RNTCP-A prospective study.
- 2001: Evaluation of yield of extra – pulmonary isolates using MB/Bact-240 system in collaboration with SDS Sanitorium & St. Marthas Hospital Bangalore.
- 2002: Effectiveness of two models of DOTS providers under RNTCP in BMP.
- 2003: Initial drug resistance among tuberculosis patients under DOTS programme in Bangalore city.
- 2003: Accessibility and utilization of anti – TB services by slum dwellers of Bangalore.
- 2003: Surveillance of drug resistance in Range Reddy District a collaborative study with department of International development, Blue Peter Research Centre and State TB Demonstration Centre, Hyderabad .
- 2003: Study to evaluate diagnosis of tuberculosis among HIV – infected persons – a collaborative study with Bangalore Medical College, Bangalore.
- 2003: Prevalence of Anti-Tuberculosis Drug Resistance in the Districts of Mayurbhanj (Orissa) Hoogly(W.Bengal) and Nagaon (Assam).
- 2004: Defaults among tuberculosis patients treated under DOT in Bangalore city- A search for solution.
- 2005: Multicentric evaluation of smear microscopy technique for the detection of acid-fast bacilli in sputum specimens (WHO Project).
- 2005: A simplified LQAS based random blinded crosschecking procedure for external quality assessment of AFB smear.
- 2005: Infectiousness of smear positive pulmonary tuberculosis patients.

2006: Drug resistance surveillance of Tuberculosis in the State of Maharashtra.

2007: Health assessment of captive Asian elephants in India, with special reference to Tuberculosis.

#### References

1. Padmanabha Rao K, Nair SS, Cobbold N & Naganathan N: Some operational factors influencing the utility of culture examination in the diagnosis of pulmonary tuberculosis. *Indian J TB* 1966, 13, 61-76
2. Challu VK, Mahadev B, Rajalakshmi R & Chaudhuri K: Recovery of tubercle bacilli from urine of pulmonary tuberculosis patients and its comparison with the corresponding sputum isolates. *Indian J TB* 1989, 36, 107–111.
3. Sujata Chandrasekaran, M M Chauhan and N Parimala: Serodiagnosis of Pulmonary Tuberculosis and evaluation of two ELISA kits: *Indian J TB* ,1996,43,159-162.
4. Sophia Vijay, Balasangameshwara VH & Srikantaramu N: Treatment dynamics and profile of tuberculosis patients under the District Tuberculosis Programme (DTP) – A prospective cohort study, *Indian J TB* 1999, 46, 239-49.
5. M M Chauhan, B Mahadev, V H Balasangameshwarw and N Srikantaramu: Assessment of Trisodium Phosphate for the storage and Isolation of Mycobacteria in a single step culture method. *Indian J TB* 1999,46,29-36.
6. B Mahadev, N Srikantaramu, P James, P G Mathew and R Bhagirathi: Comparison between Rapid colorimetric Mycobacterial isolation and susceptibility testing method and conventional method using LJ-Medium. *Indian J TB* 2001, 48, 129–134.
7. B Mahadev , P Kumar ,SP Agarwal, LS Chauhan and N Srikantaramu; Surviellance of Drug Resistance to Anti-Tuberculosis Drugs in districts of Hoogli in West Bengal and Mayurbhanj in Orissa. *Indian J TB* 2005, 52, 5-10.