

## National Tuberculosis Reference Laboratory

### BACKGROUND

The National Tuberculosis Reference Laboratory (NTBRL) was established in 2006 as a resource and reference facility within the NICD and NHLS. The prime function of the NTBRL is to strengthen and support the Department of Health's National Tuberculosis Control Programme by improving the NHLS tuberculosis laboratory service through capacity building of TB laboratory services within the NHLS, providing a national TB referral service, quality assurance programmes, training and the validation and introduction of state-of-the-art technology, with emphasis on rapid diagnosis of drug resistant tuberculosis. In addition, the NTBRL plays a critical role in surveillance. The establishment of the Corporate Data warehouse has markedly improved laboratory-based surveillance of tuberculosis, in particular MDR and XDR-TB and protocol development for the 2009 National Drug Surveillance Survey has been completed.

While staffing shortages have limited activities to some extent, much has been achieved and staff eagerly awaits the completion of the spacious new NTBRL building in Sandringham.

### ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

#### STRATEGIES IN SUPPORT OF NATIONAL TB CONTROL PROGRAMME (NTBCP)

**Background:** The volumes of microscopy tests for TB increased by a further 25% during 2008, while that of cultures performed increased by 24%. These volume increases are a continuation of the patterns that developed during the previous few years (Tables 1 and 2).

**Table 1: TB microscopy - positive patients per province per year (excluding KwaZulu Natal)**

Province	Year					Total
	2004	2005	2006	2007	2008	
Eastern Cape	247 983	340 854	464 085	550 391	815 650	2 418 963
Free State	164 023	192 759	208 049	211 139	212 162	988 132
Gauteng	343 640	456 564	520 171	500 532	610 558	2 431 465
Limpopo	106 689	154 953	197 219	224 280	309 380	992 521
Mpumalanga	137 120	149 335	179 217	189 207	218 358	873 237
North West	142 698	192 843	223 662	220 263	274 882	1 054 348
Northern Cape	73 019	91 499	92 969	94 594	104 519	456 600
Western Cape	375 729	406 613	406 613	463 782	518 150	2 210 801
<b>Grand Total</b>	<b>1 590 901</b>	<b>1 985 420</b>	<b>2 331 899</b>	<b>3 063 659</b>	<b>3 063 659</b>	<b>11 426 067</b>

**Table 2: TB culture - positive patients per province per year (excluding KwaZulu Natal)**

Province	Year					Total
	2004	2005	2006	2007	2008	
Eastern Cape	35 696	47 944	70 241	102 800	147 387	404 068
Free State	18 291	24 122	30 646	38 178	38 581	150 818
Gauteng	79 264	124 319	161 157	190 078	229 805	784 623
Limpopo	5 007	6 709	9 555	14 789	19 859	55 919
Mpumalanga	12 741	13 199	15 329	20 707	33 417	95 393
North West	14 570	18 954	25 564	38 692	45 982	143 762
Northern Cape	20 477	25 460	30 914	35 629	41 996	154 476
Western Cape	125 209	146 219	168 900	191 509	227 786	859 623
<b>Grand Total</b>	<b>312 255</b>	<b>406 926</b>	<b>512 306</b>	<b>632 382</b>	<b>784 813</b>	<b>2 648 682</b>

### Quality Assurance

Quality Assurance is key to attaining internationally recognised standards of excellence. The NTBRL liaises with the Microbiology Division of the NHLS External Quality Assurance (EQA) unit who provide both a smear microscopy and TB culture proficiency programme, as well as with the MRC, supporting their culture and drug susceptibility testing programme. All NHLS laboratories are now enrolled with the above programmes

To augment TB EQA, the NTBRL introduced a quarterly smear microscopy re-checking programme in 2008. Sample sizes of smears for re-checking were calculated according to a Lot Quality Assurance Sampling (LQAS) method based on smear positivity rates and numbers of AFB-negative smears per business unit during quarterly evaluation periods. Smears with specific sequence numbers identified on a random basis using the Corporate Data Warehouse (CWH), were submitted centrally for blind re-checking by a NTBRL technologist (controller). All smears with discrepant results were re-examined by a second controller whose assessment was accepted as final. Smears were evaluated on appropriate surface area, smear thickness, quality of staining, test status (positive or negative) and quantification of degree of smear positivity and a detailed report with appropriate recommendations was sent to the business manager concerned.

Following successful pilot studies in Mpumalanga and Limpopo provinces, the programme has now been extended to the Doctor George Mukhari Hospital laboratory, Rekopane and North-West business units. The program has been well received and was able to identify areas for improvement. When requested, NTBRL staff assisted with a site visit to help identify and correct microscopy problems and retraining of laboratory staff. A plan has been developed to extend the programme to all provinces during 2009, but roll-out to all nine provinces depends on capacity as NHLS laboratories process more than 3 million TB smears annually.

As there is currently no international EQA system for TB line probe assays (LPA) the NTBRL established an interim EQA programme for LPA. LPA are currently in use in NHLS laboratories across the country for the early diagnosis of MDR-TB. DNA from susceptible, monoresistant and MDR-TB organisms as well as NTM (non tuberculosis mycobacteria) was prepared, distributed and collated. A quarterly EQA round will be distributed in this fashion until an independent EQA system is established.

A proficiency programme for the performance assessment of TB laboratories by evaluating accreditation type checklists, is in operation. Checklists identified several deficiencies in the system and corrective actions were initiated and support structures established.

The quality control activities of the NTBRL are supported and funded by the Centres for Disease

Control and Prevention (CDC). It is envisioned that these activities will be greatly expanded during the next few years.

### Automation

With ever-increasing work loads and a shortage of technical staff, automation of TB laboratory processes is becoming a necessity. A project with Logistics Technology Solutions (LTS) consultants to automate the preparation process for direct smear microscopy and TB culture is underway, while in a separate collaboration with Arum Health and the US based company Guardian Technologies, the NTBRL is participating in a project to automate smear microscopy screening through the use of digitalised imaging.

### Rapid Molecular Diagnostics: South Africa FIND Demonstration Project

The South Africa FIND Demonstration Project is a collaboration between the Foundation for Innovative New Diagnostics (FIND), South African Medical Research Council, NHLS and national and provincial Departments of Health, aimed at investigating the feasibility, impact and cost-effectiveness of implementation of rapid screening for MDR-TB using the Genotype MTBDR *plus* assay in patients at high risk of MDR-TB in a high burden setting. The project was concluded during the final quarter of 2008 and closed for enrolment. More than 20 000 patients were enrolled. Collation and reporting of the data will take place after final processing of all specimens early in 2009. Preliminary data analysis on the first 10 000 sputum samples from patients enrolled in four provinces was reported on, and submitted to the Strategic and Technical Advisory Group (STAG) of the World Health Organization (WHO). Based on this data WHO amended policy for the early diagnosis of MDR-TB patients and recommended LPA as suitable and desirable for this use.

### Development of an integrated TB/HIV patient management system

Many obstacles stand in the way of effective treatment of TB, including inaccurate patient identification with inadequate tracking of individuals migrating between clinics, as well as a lack of accurate statistics. In an attempt to address these issues, NTBRL in collaboration with the CDC and LTS consultants, is developing a TB/HIV integrated patient management system, due for completion during the final quarter of 2009.

Once developed, the prototype will be field tested in two clinics and feedback from the users will be incorporated into an improved system.

Other activities of the NHLS during the year:

- Assistance in the establishment of new TB culture laboratories in Polokwane and Ermelo
- Introduction of the Line Probe Assay testing on clinician request at selected NHLS laboratories
- Technologist training in quality assurance, microscopy, culture and rapid molecular techniques

- Submission of proposals to introduce TB technician training
- Close liaison with Sizwe hospital, with NBTRL staff attending regular weekly ward rounds/ meetings

**QUALITY ASSURANCE AND SUPPORT PROJECT FOR SWAZILAND TUBERCULOSIS LABORATORY NETWORK**

Since 2007 the NICD, under direction of Dr Adrian Puren and with technical assistance of Ms Doreen Mabe, has been involved in a quality assurance support program in clinical pathology for diagnostic laboratories in Swaziland.

At the request of CDC and the Swaziland PEPFAR team, Mrs Esther Tsheola and Ms Elsie van Schalkwyk visited the National TB referral laboratory in Mbabane and other TB lab microscopy sites in Swaziland in March 2008 to assess ways in which the NTBRL could assist the Swaziland TB laboratory network with QA aspects of their laboratory diagnostic services and treatment monitoring of TB in the country. Subsequently Esther Tsheola and Prof Koornhof made an exploratory visit to meet Swaziland officials and to assess the needs of the country's TB laboratory network and ways in which a support program could be initiated in that region. A proposal was presented to CDC with an itemized budget totalling approximately \$200 000, accompanied by a detailed work plan based on a number of visits to Swaziland by Esther Tsheola envisaged for 2009.

The proposed project will focus on quality assurance measures for improved performance and general support from the NTBRL and will include upgrading of laboratory equipment and, through mentorship and

training of laboratory staff, improve diagnostic procedures and services. Expansion of TB culture and drug susceptibility testing activities at the TB referral laboratory in Mbabane and the introduction of fluorescence microscopy on concentrated sputum samples at multiple TB microscopy sites will be priorities, while laboratory-based training at the NICD, implementation of internal quality assessment strategies for the monitoring of performance, site visits and evaluation of quality assurance activities will be important components of the project, as will be the implementation of a smear microscopy rechecking program. The ultimate aim of the project would be for the Swaziland TB laboratory network to function independently with sustained high levels of performance to meet the needs of the country's TB control program.

**SURVEILLANCE**

Access to data from NHLS laboratories performing Drug Susceptibility Testing (DST) against first- and second-line anti-TB drugs through the Corporate Data warehouse (CDW) has improved management of the TB control programme. MDR/XDR-TB data extracted from the CDW (excluding KwaZulu-Natal), is used to maintain registers utilised by provinces to identify new MDR cases in the periphery for referral to MDR centres. In addition, it has enabled the NTBRL to provide annual figures of MDR- and XDR-TB cases, which are useful for monitoring the effectiveness of the National TB Control Programme as well as for establishing strategies for TB management.

Tables 3 and 4 summarise the number of MDR- and XDR-TB patients diagnosed by the NHLS by province by year as extracted from the CDW.

**Table 3: Number of MDR-TB Patients diagnosed by the NHLS by Province per Year**

Province	Year					Total
	2004	2005	2006	2007	2008	
Eastern Cape	476	574	930	1 128	1,244	4 352
Free State	107	170	204	216	269	966
Gauteng	591	704	714	1 027	896	3,932
KwaZulu Natal	512	1 109	2 402	2 239	1,220	7 482
Limpopo	84	53	76	114	196	523
Mpumalanga	153	123	144	473	553	1 446
North West	133	194	213	390	269	1 199
Northern Cape	155	142	178	194	178	847
Western Cape	1 187	1 183	1 204	1 459	903	5 936
<b>Total</b>	<b>3,398</b>	<b>4 252</b>	<b>6 065</b>	<b>7 240</b>	<b>5 728</b>	<b>26 683</b>

**Table 4: Number of XDR-TB patients diagnosed by the NHLS by province per year**

Province	Year					Total
	2004	2005	2006	2007	2008	
Eastern Cape	10	15	66	106	203	400
Free State	2	4	4	6	7	23
Gauteng	37	13	19	43	35	147
KwaZulu Natal	50	213	322	228	155	968
Limpopo	1	0	3	0	2	6
Mpumalanga	0	0	1	11	6	18
North West	6	10	15	10	10	51
Northern Cape	6	8	3	7	11	35
Western Cape	27	21	29	43	51	171
<b>Total</b>	<b>139</b>	<b>284</b>	<b>462</b>	<b>454</b>	<b>480</b>	<b>1 819</b>

Further development of the CDW is planned for patient specific data, enabling longitudinal follow-up of patients and identification of programme indicators at all levels.

The NTBRL performed second-line DST on ~700 samples for the Tuberculosis Drug Resistance Survey, Khayelitsha, Cape Town. The primary objective of the study was to estimate the prevalence MDR- and XDR-TB among new and re-treatment cases in Khayelitsha and to formulate recommendations for adoption of the standard treatment regimen for drug-resistant TB used in that area.

Protocol development for the National Drug Surveillance Survey has been completed. Population-proportionate cluster sampling ensures specimens are representative of TB patients in South Africa. Fieldwork commences in 2009. Prevalence of MDR/XDR in new and re-treated cases will be established and used in rational management planning.

**COMMUNITY ACTIVITIES**

In October 2008, despite heavy routine work loads, several staff members participated in MegaCARE held at the NASREC centre in Johannesburg. MegaCARE, a PEPFAR initiated event, was a new component of the spiritual event MegaFest International and aimed at providing free health care and educational opportunities for South Africans. Aided by the Department of Health, volunteers distributed information and screened sputum samples from almost 400 community members for tuberculosis.

**COLLABORATIONS**

Professor Martin Grobusch's Infectious Diseases Unit of the NHLS and School of Pathology, Faculty of Health Sciences, University of the Witwatersrand in collaboration with Sizwe Hospital staff and the NTBRL plans to study interferon- $\gamma$  release assay (IGRA) technology for the monitoring of TB acquisition in health care workers.

Guardian Technologies and Arum Health collaborate with the NTBRL in a project to evaluate digital imaging technology for improved smear microscopy and possible automation.

The Centres for Disease Control and Prevention contributed to the financing of several projects, including the development of the integrated TB/HIV management system, automation of processing of specimens for smear microscopy, the slide re-checking EQA programme and others.

Several institutions including the South African National Department of Health, CDC South Africa, NHLS, NICD, WHO AFRO, USAID, Becton Dickenson (US), ASM and JICA either fund or are involved in the running of the newly established African Centre for Integrated Laboratory Training (ACILT).

The South Africa FIND demonstration project is a collaboration between FIND, South African Medical Research Council, NHLS and national and provincial Departments of Health, aimed at investigating the feasibility, impact and cost-effectiveness of implementation of rapid screening for MDR-TB using the Genotype MTBDR *plus* assay in patients at high risk of MDR-TB in a high burden setting.

NHLS collaborates with the Department of Health on an ongoing basis to improve laboratory services and patient management and members of the NTBRL staff liaise regularly with staff at Sizwe hospital.

The NTBRL established collaboration with Prof Gilla Kaplan's team from the International Center for Public Health, New Jersey, USA in a cross-sectional population-based study aimed at exploring host and pathogen factors that contribute to the failure of treatment of MDR-TB, ultimately leading to the emergence of XDR-TB strains. The identification of factors contributing to poor treatment outcomes in MDR-TB, could be used in forming future TB control strategies.

## **CAPACITY BUILDING**

### **AFRICAN CENTRE FOR INTEGRATED LEARNING (ACILT)**

Given the shortages of trained staff and their vital role in the development and maintenance of excellent laboratory services, a highlight of 2008, was the establishment of ACILT.

ACILT was established in 2008 on the Johannesburg campus of the South Africa National Institute for Communicable Diseases (NICD) and the National Health Laboratory Service (NHLS). The aim of this new centre is to build a new generation of laboratory experts, particularly in the fields of HIV, TB and malaria throughout Africa. The vision of ACILT is *“a healthier Africa through quality laboratory practices that support efforts to combat major infectious diseases”*, while the mission is *“to provide integrated hands-on training courses to expand laboratory capacity in Africa for the diagnosis and monitoring of major infectious diseases including HIV/AIDS, TB and malaria.”*

Various partners contributed to the establishment of ACILT, and some will partake in the governance of the centre. Stakeholders include the South African National Department of Health, CDC South Africa, NHLS, NICD, WHO AFRO, USAID, Becton Dickenson (US), ASM and JICA. Funding of the centre will be by PEPFAR through the CDC South Africa.

Under the auspices of the newly appointed director, Ms Elsie van Schalkwyk, courses will be focussed on TB culture/DST, microscopy and molecular diagnostics, HIV Early Infant Diagnosis PCR, BED, laboratory management, quality management systems (QMS) and commodity management.

To date 5 courses have been offered by ACILT to 85 participants, while a further 14 courses to an estimated 200 attendees will be presented during 2009. Participants included laboratory staff from Nigeria, Uganda, Ethiopia, Mozambique, Namibia, Angola, Rwanda, Botswana, Malawi, Ivory Coast, Tanzania, Zambia, Zimbabwe and South Africa.