



Special Programmes

TB Focus Programme & National Tuberculosis Reference Laboratory

BACKGROUND

The National Tuberculosis Reference Laboratory (NTBRL) was established in 2006 as a reference and resource facility within the NICD and NHLS. Its prime function is to support the National Tuberculosis Control Program (NTBCP) of the Department of Health and its mission is to act as an integrated force within the NHLS, driving the tuberculosis diagnostic services in the public health sector to internationally recognised standards of excellence through quality assurance, service-oriented research and training. In the process the NTBRL aims to introduce cost-effective, state-of-the-art technology practiced by efficient, well-equipped staff that act professionally and with integrity.

Core NTBRL staff were appointed in 2006 and 2007. A modern building specifically designed for the NTBRL will be completed in 2008 and in the meantime technical and research staff members were housed at the NHLS TB laboratory and in a newly established molecular laboratory, both on the NHLS Braamfontein campus. Other staff moved into NICD offices in Sandringham.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

STRATEGIES IN SUPPORT OF NATIONAL TB CONTROL PROGRAM

In order to support the NTBCP, the NTBRL prioritized the reorganization of laboratory services to shorten turn-around times and utilize molecular techniques for the rapid identification of multidrug-resistant tuberculosis (MDR-TB) cases.

The NTBRL liaised closely with the Microbiology Division of the External Quality Assessment (EQA) Unit of the NHLS in support of their proficiency testing program of smear microscopy and TB culture, as well as with the MRC proficiency testing program for culture and drug susceptibility testing (DST). To augment TB EQA, NTBRL plans an extensive smear microscopy rechecking program to commence in 2008. The Centers for Disease Control and Prevention (CDC) actively support the NHLS EQA activities and envisage an expanded proficiency testing role for the NTBRL in African countries. The CDC also aims to alleviate the administrative burden of EQA programs through development of web-based systems and utilization of fax/scan technologies.

Seminars, workshops and regular meetings were held to discuss and monitor progress of strategies pursued in close collaboration with national and provincial TB programs. Topics covered in 2007 were:

- Quality and turn around times of sputum smear microscopy
- Specimen and report tracking
- Improved capacity for DST and optimal utilization of drug susceptibility data, including institution of drug resistance surveys for South Africa, Lesotho and Swaziland.
- Integration of TB and HIV/AIDS laboratory-based management at clinic and other levels
- Information sharing on upgrading of laboratories and new developments in TB diagnosis
- Quality management and standardization issues
- Data provision from MDR-TB and XDR-TB registers for longitudinal follow-up of patients
- Activities and progress to redress problems in identified crisis regions
- Collaborative epidemiological studies, including involvement in outbreak management and molecular epidemiology-based studies.
- Training on all levels to increase knowledge base and improve treatment literacy. Refresher courses will be aimed at medical technologists and technicians, focusing on microscopy, culture, DST, rapid molecular techniques, and training in quality management. Establishment of an African Centre for Integrated Laboratory Training (ACILT) is being planned.

MDR- AND XDR-TB CASES FROM NHLS DATA BASE

Access to data from NHLS laboratories performing DST against first- and second-line anti-TB drugs, enabled NTBRL to provide annual figures of MDR-TB and XDR-TB cases (Table 1).

Within a large pool of MDR-TB cases (7369 new cases in 2007), escalating numbers of XDR-TB cases emerged with 536 new cases occurring in South Africa in 2007. By far the majority of XDR-TB cases were in KwaZulu-Natal, followed by the Eastern Cape, Gauteng and Western Cape.

Table 1: MDR-TB and XDR-TB cases detected annually in provinces by NHLS laboratories during period 20042007

Year	KZN ^a		EC ^b		GP ^c		WC ^d		Other ^e		All	
	MDR	XDR	MDR	XDR	MDR	XDR	MDR	XDR	MDR	XDR	MDR	XDR
2004	507	44	478	11	608	30	1205	23	634	9	3432	117
2005	1127	217	580	11	723	4	1199	11	694	19	4323	252
2006	2445	318	952	61	727	19	1239	12	836	29	6199	439
2007	2229	216	1187	107	1075	53	1461	56	1490	32	7442	464
Total	6308	795	3197	190	3133	106	5104	102	3654	89	21396	1282

^a KwaZulu-Natal; ^b Eastern Cape; ^c Gauteng Province; ^d Western Cape; ^e Other: North-West Province MDR 825, XDR 31; Mpumalanga MDR 829, XDR 7; Northern Cape MDR 704, XDR 16; Free State MDR 660, XDR 9; Limpopo MDR 355, XDR 16

EVALUATION OF THE GenoType® MTBDRplus ASSAY FOR RAPID DETECTION OF MULTIDRUG-RESISTANT TUBERCULOSIS FROM SMEAR-POSITIVE SPUTUM SPECIMENS

Pilot studies were conducted in Johannesburg and Cape Town, employing the line probe GenoType® MTBDRplus assay (Hain Lifescience, GMBH, Nehren, Germany), labeled MTBDRplus method, for rapid detection of resistance to rifampicin and isoniazid. The design of the studies was similar and PCR amplification followed by detection of gene mutations by line probe was performed on smear-positive sputum samples which were also subjected to DST by MGIT 960.

In Johannesburg 730 and Cape Town 356 sputum samples processed by both methods were analysed. Concordance was excellent (see Table 2 for Johannesburg findings) and the sensitivity, specificity, and positive and negative predictive values for

detection of MDR cases in both studies were high (~98% - 100%). Exceptions included sensitivities of 92.2% and 94.2% for detection of isoniazid resistance in the respective studies, reflecting failure of MTBDRplus to detect isoniazid resistance in 7(7.8%) out of 90 and 7(6.1%) out of 114 specimens in Johannesburg and Cape Town respectively. Failure to detect rifampicin resistance also occurred: 1 in Cape Town and 5 (sensitivity of MTBDRplus 93.7%) in Johannesburg, while 2 falsely resistant rifampicin findings were recorded in each of the 2 studies.

Contamination rates were 19% and 15.4% respectively for Johannesburg and Cape Town. Although DST results were not available for comparison with MTBDRplus findings in contaminated specimens, the ability of MTBDRplus to produce interpretable results in most specimens was an important feature of the method.

Table 2: Rapid identification of multidrug-resistant TB in sputum specimens by the GenoTypeMTBDRplus PCR assay compared with DST by the MGIT 960 system as "gold standard" amongst 730 *M. tuberculosis* smear-positive specimens: Johannesburg study

Method	Number of <i>M. tuberculosis</i> isolates						Total
	Non-MDR		MDR		Falsely	Falsely	
	+ve	Concordant ^a	+ve	Concordant ^a	Non-MDR ^b	MDR ^b	
MGIT	661	661	69	68	-	-	730
PCR	662	661	68	68	1	0	730

^aSusceptible and resistant findings between the two methods identical.

^bFalsely non-MDR-TB or MDR-TB compared with "gold standard" MGIT 960 system DST findings.

Table 3: Analysis of concordance between BACTEC 460 and MGIT 960 systems

Drug/s Tested	Number of assays	Concordance (%)	Discrepancies (%)	Falsely resistant (%)	Falsely susceptible (%)
ETH ^a	88	80 (91)	8 (9.1)	6 (6.8)	2 (2.3)
KAN ^b	88	87 (99)	1 (1.1)	1 (1.1)	0 (0)
OFX ^c	88	84 (95)	4 (4.5)	4 (4.5)	0 (0)

^a ETH ≤ Ethionamide; ^b KAN ≤ Kanamycin; ^c OFX ≤ Ofloxacin



Esther Tsheola and Zaheda Bhyat doing drug susceptibility testing on the MGIT machine.

VALIDATION OF THE BACTEC MGIT 960 SYSTEM FOR SUSCEPTIBILITY TESTING OF THREE SECOND-LINE ANTI-TB DRUGS COMPARED WITH THE RADIOMETRIC BACTEC 460 TB SYSTEM

As backlogs of cultures ear-marked for second-line DST occurred, an in-house evaluation of the performance of the MGIT 960 compared with BACTEC 460TB system was conducted for susceptibility testing of ethionamide, kanamycin and ofloxacin on 138 cultures of varying ages and resistance profiles (33 were XDR-TB strains). Concordance between the two methods, shown in Table 3, was excellent: 99% for kanamycin, 95% for ofloxacin and 91% for ethionamide. Of 13 (14.8%) discrepancies amongst 88 evaluable cultures, 11 were falsely resistant. Five cultures with discrepancies involving kanamycin and ofloxacin were all falsely resistant. As a result, 1 patient would have been labeled an XDR-TB case due to a falsely resistant kanamycin finding.

Significantly more cultures stored for 10 weeks or longer were either contaminated (20 out of 91; 22.0%) or were rejected by the MGIT 960 system because of poor growth (5 cultures), compared with 4 out of 47 (8.5%) contaminants in the ≤10 age group ($p \leq 0.02$).

COLLABORATIONS

Under the auspices of the Foundation for Innovative New Diagnostics, South African Medical Research Council and National Department of Health, a proof of concept study to evaluate the performance of the **GenoType®MDR-TBplus** (Hain Lifescience, GMBH, Germany) assay for the rapid diagnosis of MDR-TB in 20000 smear microscopy-positive TB patients in Johannesburg, Cape Town, Durban and Kimberley was started in June 2007, after highly satisfactory findings were obtained in two pilot studies (See earlier section).

The NTBRL collaborated with the Department of Health on patient management issues, especially related to XDR-TB, including guidelines for its containment. Members of the NTBRL were, together with other experts from Gauteng hospitals, part of an XDR-TB management team at Sizwe Hospital.

The Centers for Disease Control and Prevention contributed to the financing of capital equipment for the new NTBRL building and provided useful advice on design features of the laboratory. It is also playing a supportive role in the TB proficiency testing programme of the NHLS.



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CAPACITY BUILDING

The NTBRL established 144 new smear microscopy sites between 2005 and 2007 throughout the country, while several culture laboratories were added to the expanded TB diagnostic network of the NHLS. All new staff at these sites received training by experienced NHLS staff. Specialized training of recently appointed technologists and medical scientists has either been provided by experienced local staff or when required, new staff will receive specialized training at appropriate local or overseas institutions. Plans to this effect are already in place.