

Experience establishing tuberculosis laboratory capacity in a developing country setting

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SUMMARY

OBJECTIVE: To describe the experience of strengthening laboratory diagnosis of tuberculosis (TB) in a resource-limited country with high TB-HIV (human immunodeficiency virus) and multidrug-resistant TB (MDR-TB) prevalence.

METHODS: In the Kingdom of Lesotho, which is confronted with high levels of TB, MDR-TB and HIV prevalence, between 2006 and 2008 a coalition of the Foundation for Innovative New Diagnostics, Partners In Health and the World Health Organization renovated the National TB Reference Laboratory and reinforced microscopy services, streamlined conventional culture and drug susceptibility testing (DST) and introduced modern TB diagnostic methods.

FINDINGS: It was feasible to establish a biosafety level three facility for solid culture and DST and an external quality assessment programme for smear microscopy within 4 months, all in 2007. Liquid culture and DST

were introduced a month later. Preliminary results were comparable to those found in laboratories in industrialised countries. A year later, line-probe assay for the rapid detection of MDR-TB was introduced.

DISCUSSION: Through strong political commitment and collaboration, it is possible to rapidly establish quality assured TB diagnostic capacity, including current methods, in a resource-limited setting. Case detection and management for TB and MDR-TB have been greatly enhanced. From a low baseline, TB culture throughput in the laboratory increased ten-fold and has been sustained. This experience has served as a catalyst to translate policy into practice with new diagnostic technologies. It supports global policy setting to enhance and modernise laboratory work in developing countries.

KEY WORDS: TB laboratory capacity; liquid culture; line-probe assay; MDR-TB; low-income country

TUBERCULOSIS (TB) continues to be one of the most serious challenges facing health systems in developing countries, with an estimated 9.2 million new cases and 1.7 million deaths in 2006.¹ The incidence of multidrug-resistant (MDR) TB has been increasing, with an estimated 489 139 cases emerging in 2006.² The increase in MDR-TB, along with reports of extensively drug-resistant (XDR) TB, has led to the development of a global response plan to address the serious threat of drug-resistant TB.³ An important component of this plan calls for significant expansion of TB diagnostic laboratory capacity, upon which the detection of MDR-TB and XDR-TB depends. The required increase in the scope of laboratory services in high-burden countries has been estimated to include up to 13 000 centres for smear microscopy, 130 advanced diagnostic centres for culture and drug susceptibility testing (DST), five national referral laboratories, and an increase in the number of supranational reference laboratories. The

challenge of drug resistance is further amplified in countries where there is also a high rate of human immunodeficiency virus (HIV) infection.

The Foundation for Innovative New Diagnostics (FIND) is a non-profit Swiss organisation launched at the 56th World Health Assembly in 2003 to accelerate and drive the development process of diagnostic tests for poverty-related diseases, and to evaluate and demonstrate the efficacy and effectiveness of these tools under typical field settings in developing countries. The diagnostics FIND has worked on include liquid culture for TB and DST, a rapid immunoassay for species identification of *Mycobacterium tuberculosis* complex strains from culture isolates, and a molecular line-probe assay (LPA) for detection of isoniazid (INH) and rifampicin (RMP) resistance within 48 h directly from sputum samples. Evidence from large-scale clinical trials was submitted to the World Health Organization (WHO) Strategic and Technical Advisory Group for TB (STAG) in 2007

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and 2008. Following a systematic review of data on these assays by independent experts, the WHO endorsed these technologies for use in TB laboratories in developing countries.^{4,5} However, rolling out the new technologies on a wider scale is only feasible if the laboratories in high-burden countries are strengthened and prepared in terms of infrastructure, quality assurance (QA) systems, standard operating procedures (SOPs) and training. In view of existing limitations, FIND has established a laboratory support programme to demonstrate and document the steps needed to translate policy into practice. A project conducted in Lesotho represented an early opportunity to respond to the urgent need to establish TB diagnostic capacity in low-resource settings.

METHODS

A small landlocked country with limited natural resources and a population of approximately 2 million people, Lesotho is affected by both the HIV and TB epidemics, with an estimated HIV prevalence of 25% among adults aged 15–49 years and a case notification rate of 605 TB cases per 100 000 population.^{1,6} As of 2006, however, TB diagnostic capacity within the National TB Programme (NTP) was limited to smear microscopy, performed in 17 microscopy centres without an adequate QA programme. All clinical specimens from MDR-TB suspects requiring culture and DST had to be sent out of the country at high cost to laboratories in either South Africa or the United States. In October 2006, the Ministry of Health (MoH) of Lesotho requested assistance through the WHO for strengthening TB diagnostic services.

In response, a team from FIND made a laboratory assessment visit to the National TB Reference Laboratory (NTRL) located at Queen Elizabeth II Hospital in Maseru, the capital of Lesotho, in November 2006. The team found that significant renovation of the laboratory was needed, essential equipment for DST was missing, and that a QA programme for smear microscopy was only partially implemented (Figure 1). It was evident that resolving these issues could best be accomplished in partnership with other agencies, to upgrade laboratory capacity at minimal cost. Several partners were rapidly identified, principally Partners In Health, which was in the process of establishing a treatment programme for MDR-TB in Lesotho, and the WHO. FIND provided a full-time onsite consultant and procurement of an instrument for automated TB liquid culture and DST, in addition to a continuous supply of reagents. A multiphase work plan was drawn up for correcting the deficiencies.

In the first phase, which covered the period from May to August 2007, the Lesotho NTP and other stakeholders collaborated to develop training modules and accompanying manuals to bring the performance of sputum microscopy up to quality standards. Labo-



Figure 1 Tuberculosis reference laboratory, pre renovation.

ratory personnel were given refresher training in smear microscopy. A QA programme was put in place for smear microscopy, consisting of onsite evaluation and supervision and random blinded rechecking of slides following standard guidelines.⁷ At first, this consisted of re-examination of 15% of all slides. Based on the data from this exercise and follow-up trainings as needed, Lot Quality Assurance Sampling (LQAS) was put in place for external quality assessment (EQA) of smear microscopy across all centres starting in September 2008. EQA included onsite evaluation and random blinded rechecking every month of all sites except two, where it was done less frequently for logistical reasons. Panel testing is being carried out periodically with slides obtained from the Supranational TB Reference Laboratory (SNRL) in Pretoria, South Africa.

In parallel, the NTRL was renovated with the creation of a biosafety level three (BSL3) facility that would meet the WHO-recommended requirements for handling liquid TB culture (Figure 2).⁸ TB solid culture and DST were implemented, with EQA provided by the SNRL, South Africa.

With this basis for the activities in the first phase, TB liquid culture and DST, along with rapid immunoassay-based species identification, were introduced in the second phase. Isolation and contamination rates for solid culture on Löwenstein-Jensen (LJ) media and TB liquid culture using the BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960™ TB System (BD Diagnostic Systems, Sparks, MD, USA) were available by December 2007.

In the third phase, activities to prepare for the introduction of the LPA for detection of MDR-TB began with the construction of a clean-room facility in July and August 2008. The introduction of the assay and training of laboratory staff took place in October 2008.

RESULTS

Through the transformative efforts undertaken, TB laboratory diagnostic capacity at the NTRL increased



Figure 2 Tuberculosis reference laboratory, post renovation.

dramatically, from fewer than 100 TB cultures per month to more than 700 cultures per month by June 2008 (Figure 3).

Validation of the liquid culture method took place from December 2007 to February 2008, and revealed contamination rates of respectively 1.9% and 7.8% for solid and liquid TB culture (Table). *M. tuberculosis* isolation rates, as confirmed by the lateral flow

Table Validation results for LJ vs. MGIT

LJ	MGIT				Total
	Culture-positive	Culture-negative	NTM	Contaminated	
Culture-positive	77	3	2	6	88
Culture-negative	14	227	1	19	261
NTM	1	0	1	1	3
Contaminated	1	4	0	2	7

LJ = Löwenstein-Jensen; MGIT = Mycobacteria Growth Indicator Tube; NTM = nontuberculous mycobacteria.

immunochromatographic assay (Capilia TB, TAUNS, Numazu, Japan),⁹ and excluding contaminated specimens, were respectively 22.3% and 25.3%, showing a relative increase of 14% in sensitivity of liquid culture compared to solid culture.

Between January 2008 and March 2009, 8569 specimens were processed for culture, including the use of both LJ and MGIT, with an overall contamination rate of 10.8% (data not available to permit the separate calculation of contamination results for both methodologies). Considering all smear-positive cases, 87% of the samples were culture-positive. The smear-positive, culture-negative rate, calculated as smear-positive/culture-negative divided by total number of cultures, was low, at 104 (1.6%), and most of these samples were from follow-up cases. However, the rate of smear-negative, culture-positive results, calculated as smear-negative/culture-positive divided by total culture-positives, was high, at 538 (49.9%), which is not surprising considering the alarmingly high rate of HIV positivity among TB patients (Figure 3).

After validation and retraining, LPA has started to be used on a routine basis in addition to liquid culture for MDR-TB suspects. An interim analysis at 3 months shows a concordance of 80% (32/40) on

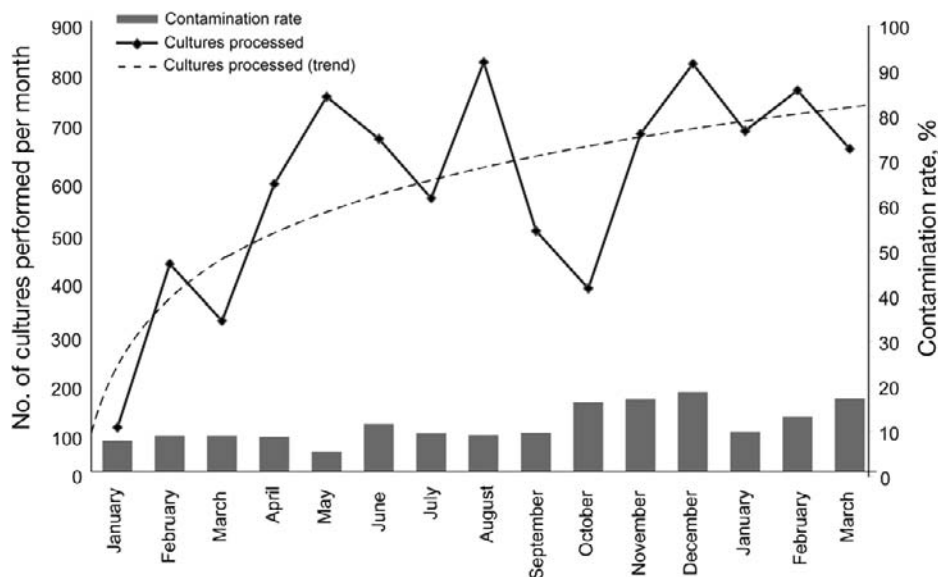


Figure 3 Number of cultures processed and contamination rates.

direct sputum samples. The discordant samples are undergoing more detailed analysis, including sequencing and repeat DST on solid and liquid culture. Re-training was also undertaken in April 2009, and on-site supervision to evaluate various results in June 2009.

In terms of microscopy, an estimated 900 smears were being examined each month at the NTRL prior to the intervention. After the strengthening of smear microscopy, the total number of smears examined, as reported by 14 microscopy centres and the NTRL, had reached 85 471 slides, representing specimens from 34 955 patients. Of these, 33 473 slides, representing 14 372 patients, were examined at the NTRL. The overall positivity rate was 11.7%. The false-positive and false-negative rates from the EQA programme were both <1%.

The investment required to achieve this dramatic turnaround of the TB diagnostic services in Lesotho was less than US\$550 000, including US\$93 000 for laboratory infrastructure upgrade, US\$65 000 for TB diagnostic instruments, excluding the ones that were available but unused, US\$280 000 for reagents and consumables for one year, and \$90 000 for human resources during the project. A detailed report on the costing is being published separately.

DISCUSSION

Laboratory capacity to properly diagnose and manage MDR-TB is an essential component of the multi-faceted response to the urgent challenges posed by MDR- and XDR-TB. This situation is compounded by the current state of laboratories in many developing countries that also suffer from the highest burdens of TB and HIV. In many of these countries, weaknesses in delivery systems for diagnostic services include deficits in laboratory infrastructure, overreliance on light microscopy, outdated SOPs, absence or lack of QA, and a shortage of trained personnel. Diagnostic services have been among the most neglected sectors of the health system.

The development of new diagnostic tools for TB and the demonstration of their effectiveness in the field offer great promise. However, these tools have to be translated into NTP policy and implemented in laboratory services to play an effective role in improving TB and MDR-TB case detection and management. There is no clear road map for ensuring the uptake of newer tools, as existing diagnostic tools for TB, such as light microscopy, solid culture and solid culture-based DST, have been in use for many decades. Furthermore, in developing countries, resources available to laboratories in terms of infrastructure, trained personnel and financing vary between the different levels of the TB laboratory network within a given country and from country to country. For example, while India has a population

500 times greater than that of Lesotho, with an estimated 12 000 centres for TB microscopy, the country's NTP has succeeded in establishing a QA programme that covers more than 75% of microscopy centres. However, India still faces the challenge of establishing solid TB culture and DST in 26 intermediate reference laboratories (IRLs) at the state level, and currently performs these tests in only four NTRLs and eight IRLs.¹

Although the whole process of upgrading TB laboratory diagnostic services in Lesotho as described above was accomplished within a relatively short time period, there were challenges along the way that resulted in lessons being learned.

First, it was necessary to secure political will and commitment, with full buy-in to the process at the highest levels of the MoH as well as by the NTP, the WHO, and other partners already working in the country. Second, ensuring the reliable supply of reagents and consumables was at times challenging, and required constant vigilance to ensure timely customs clearance and sufficient lead time in ordering. The very success of the laboratory quickly led to high expectations and increased demand on the laboratory for diagnostic testing, stretching the limits of the original facility and laboratory personnel. The high profile of the project also resulted in frequent partner visits, and requests for hands-on onsite training for technicians from other African countries, which at times was stressful for the staff who were busy trying to keep up with the workload, and who missed out on some training opportunities. This made it challenging to maintain contamination rates within acceptable limits.

Longer term sustainability requires that TB diagnostic services be integrated into a national laboratory policy and strategic implementation plan. This is reflected in the Maputo Declaration of 2008,¹⁰ which emphasises the importance of addressing the diagnostic needs of all disease control strategies of public health importance, including TB, and calls on donors and implementing partners to provide support for laboratory strengthening with a view to ensuring national ownership. Ideally, this should pave the way to ensuring steady financing through national resources. Although, at the current time, Lesotho is not alone among resource-limited countries in its current requirement for support from external financing via donors, the collaborative nature of this project has helped ensure that no single source of financing has had to bear the entire cost, and that multiple partners will be working together to cover the financial needs. In Lesotho, the commitment of the MoH and partners to collaborate with complementary resources enabled TB diagnostic services in the country to rapidly be brought up to the standards of the developed world. Further support is being provided by the WHO, who led an international team of experts in a site visit

in early July 2008 and devised a work programme to further update the SOPs based on current (updated) WHO recommendations.

Together with partners, it is urgent to build on the experience in Lesotho and the lessons learned to translate WHO policy on TB diagnosis into practice in settings with different challenges and capacity. New mechanisms for coordination of partners have been instituted, including the WHO Global Laboratory Initiative (GLI), which was established to bring partners together to address the challenges of upgrading laboratory services to meet the needs of the MDR-TB and XDR-TB epidemics and other common infectious diseases. The US President's Emergency Plan for AIDS Relief (PEPFAR), through the Centers for Disease Control and Prevention (CDC) Global AIDS Program, has made a major commitment to supporting laboratory strengthening in many countries over the next 5 years. UNITAID has provided an initial grant to a coalition of partners, including FIND, GLI and the Global Drug Facility of the Stop TB Partnership, to implement new TB diagnostic technologies in up to 16 high-burden countries over the next 3 years. Current plans are underway to expand this project to a total of 27 countries.

The speed with which WHO policy on TB diagnostics, developed with the active involvement of many organisations such as the International Union Against Tuberculosis and Lung Disease, the CDC, the United States Agency for International Development and the Association of Public Health Laboratories, among others, is being translated into effective use, and uptake through these and other activities offers a new road map for the adoption and implementation of new health innovations for public health. Past experience has shown that many years often elapse between policy recommendations and the uptake of new products in the field. However, the experience in Lesotho has played an important role in demonstrating that it is possible to improve health systems without long delays when concerted efforts bring together new technologies and partners in a common cause.

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RÉSUMÉ

OBJECTIF : Décrire une expérience de renforcement du diagnostic de laboratoire de la tuberculose (TB) dans un pays à ressources limitées où la prévalence de TB-VIH (virus de l'immunodéficience humaine) et de TB multi-résistante (TB-MDR) est élevée.

MÉTHODES : Dans le Royaume du Lesotho, qui est confronté à des niveaux élevés de prévalence de la TB, de la TB-MDR et du VIH, une coalition de la Foundation for Innovative New Diagnostics, Partenaires en Santé et

l'Organisation mondiale de la Santé, a rénové le laboratoire national de référence de TB entre 2006 et 2008 et renforcé les services d'examen microscopique, rationalisé les cultures conventionnelles et les tests de sensibilité (DST) aux médicaments et introduit des méthodes modernes de diagnostic de la TB.

RÉSULTATS : Il a été possible de mettre en place un service de biosécurité de troisième niveau pour la culture sur milieu solide et les tests de sensibilité ainsi qu'un

programme de contrôle de qualité externe de l'examen microscopique des frottis au cours de 4 mois pendant l'année 2007. Un mois plus tard, on a introduit la culture sur milieux liquides et les DST. Les résultats préliminaires ont été similaires à ceux observés dans les laboratoires des pays industrialisés. Une année plus tard, on a introduit un test « line probe » pour la détection rapide de la TB-MDR.

INTERPRÉTATION : Grâce à une implication et à une collaboration politique puissante, il est possible de mettre rapidement en place dans un contexte à ressources limi-

tées un diagnostic de TB de qualité garantie comportant les méthodes courantes. On a renforcé considérablement la détection des cas et la prise en charge de la TB et de la TB-MDR. A partir d'un point de départ bas, le débit des cultures TB au laboratoire a été multiplié par dix et a été maintenu. Cette expérience a servi de catalyseur pour le passage du stade politique au stade pratique en ce qui concerne les nouvelles technologies de diagnostic. Elle plaide en faveur de la mise en place d'une politique globale par le renforcement et la modernisation du travail de laboratoire dans les pays en développement.

RESUMEN

OBJETIVO: Describir la experiencia del fortalecimiento del diagnóstico de laboratorio de la tuberculosis (TB) en un país con recursos limitados y una alta prevalencia de TB, coinfección por el virus de la inmunodeficiencia humana (VIH) y TB multidrogorresistente (TB-MDR).

MÉTODOS: En el Reino de Lesoto, que presenta una alta prevalencia de TB, TB-MDR e infección VIH, una coalición de la Fundación para Nuevos Diagnósticos Innovadores (FIND), Partners In Health y la Organización Mundial de la Salud renovó el laboratorio nacional de referencia de TB y reforzó los servicios de microscopia, reestructuró los cultivos convencionales y los antibiogramas e introdujo métodos modernos de diagnóstico de la TB entre el 2006 y el 2008.

RESULTADOS: En un período de 4 meses en el año 2007, fue factible organizar instalaciones con bioseguridad de tercer nivel destinadas a los cultivos de micobacterias en medio sólido y los antibiogramas y se estableció un programa de control externo de la calidad de las baciloscopias. Un mes más tarde, se introdujeron los cultivos y las

pruebas de sensibilidad en medio líquido. Los resultados preliminares fueron comparables a los resultados obtenidos en laboratorios de países desarrollados. Las pruebas de diagnóstico rápido de la TB-MDR mediante ampliación genómica con hibridación reversa en tiras (LPA) se pusieron a punto un año después.

CONCLUSIONES: El firme compromiso político y la colaboración hacen posible, en los entornos con recursos limitados, establecer en forma rápida una estrategia de diagnóstico de la TB por los métodos corrientes, con un sistema de control de la calidad. Se fortaleció considerablemente la detección de casos y el tratamiento de la TB-MDR. Se aumentó en diez veces la baja capacidad inicial de cultivo de micobacterias y se logró sostener este nivel de actividad. La presente experiencia ha servido como un catalizador de la traducción de las políticas a la práctica en materia de nuevas técnicas diagnósticas y respalda la política mundial en favor del fortalecimiento y la modernización del trabajo en los laboratorios de los países en desarrollo.
