

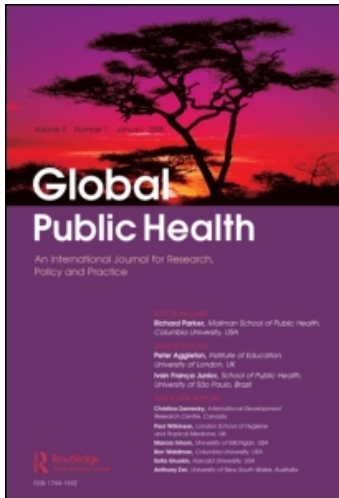
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Reversing the tide of tuberculosis in India: Complementing microscopy with line probe assays

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Abstract

In 1993, the World Health Organisation (WHO) declared tuberculosis (TB) a global health threat, adopted the Directly Observed Therapy – Short Course (DOTS) strategy, and set two targets for control and elimination of the disease: to detect 70% of sputum smear positive cases and to successfully treat 85% of those cases. The recommended diagnostic tool under DOTS remains sputum smear microscopy, a simple, yet ineffective, technique that only detects roughly half of TB cases. In India, where TB killed 450,000 people in 2005, both WHO targets for detection and treatment were met in the smear positive population covered by DOTS. However, HIV co-infection and multidrug-resistant TB (MDR-TB) pose formidable threats to TB control: TB in HIV-positive patients is often smear-negative, and microscopy cannot detect drug resistance. Although, the reliance on DOTS has proven effective in areas where both HIV prevalence and drug resistance are low, in India, the National TB Programme should consider complementing the antiquated technique of microscopy in order to diagnose smear-negative, extra-pulmonary, and MDR-TB cases. Integrating existing rapid molecular diagnostics with the Indian National TB Programme is timely, and would be extremely beneficial to address the two major threats to TB control in the country.

Keywords: *tuberculosis, diagnosis, India*

Introduction

Tuberculosis (TB), one of the world's oldest infectious diseases, has plagued humans since the 4th millennium BC (Nerlich et al. 1997). TB still claims 5000 lives every day, more than SARS (774 deaths) (World Health Organisation

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(WHO 2006c), Marburg (150 deaths) (WHO 2006b), and avian flu (76 deaths) (WHO 2006a) ever have (WHO 2006d). However, during the last 40 years, advances in the treatment and control have stopped the disease from posing a significant public health threat in the wealthy nations. Consequently, TB rarely makes the headlines. This lack of media and political attention, in part, explains the low levels of funding for TB research and development and its most telling results: diagnosis of the disease is currently still relying on a 124-year-old technique, no new drug for TB has been brought to market in the last 40 years (Zhang 2005), and BCG,¹ a largely ineffective vaccine, is still widely administered (Andersen and Doherty 2005). Recently, however, virtually untreatable, extensively drug-resistant TB (XDR-TB) strains resistant to second-line drugs, have been described, placing TB on the forefront of global public health threats (Anon 2006a, 2006b).

This paper will argue that inadequate diagnosis constitutes a particularly important obstacle to TB control in the developing world. Powerful new diagnostic tools have been developed in the recent years, but are not routinely used and are not part of the WHO recommended TB control strategy. We believe that selective use of these new technologies could transform the battle against TB, especially where drug resistance and HIV co-infection are widespread. A rapid, 100% sensitive and 100% specific test would save 105,000 lives annually if available only to those with access to advanced infrastructure, or 359,000 if rolled out to all existing TB clinics (Keeler et al. 2006). India, which suffers from the world's heaviest TB burden, and which possesses the technical capacity to make good use of the new tools, offers a particularly promising testing ground for this new approach to TB diagnosis.

Tuberculosis (TB) diagnostics

In light of TB's evolving and increasing menace in the developing countries, the WHO declared it a global health threat in 1993. Since then, however, its TB control strategy² has relied on sputum smear microscopy, an increasingly obsolete diagnostic test (Brodie and Schluger 2005). Studies on TB diagnostics largely agree: microscopy is an inaccurate tool, whose sensitivity ranges from 40 to 60% under field conditions, falling as low as 20% in the presence of HIV co-infection (Brodie and Schluger 2005, StopTB-Partnership 2006).

This leads to a series of obstacles for both the patients and the TB programmes using microscopy: under Directly Observed Therapy – Short Course (DOTS), patients provide three sputum samples, over the course of two to three days, for staining and analysis under a microscope. These multiple visits to clinics, which often require transportation fees and loss of income, can be prohibitively expensive for patients and programmes (van Cleeff et al. 2005). Exacerbating matters further, technicians are often forced to compensate for microscopy's low sensitivity by testing the same patient several times before proper diagnosis can be achieved. Furthermore, detection of bacilli under the microscope can be extremely tedious for laboratory technicians, which results in decreased accuracy.

WHO guidelines recommend that technicians examine no more than 20 samples per day in order to maintain a modicum of accuracy (WHO 2003). These guidelines are unrealistic for National TB Programmes (NTP) struggling to test a high number of patients with an ever-decreasing number of technicians.

Alternatives to microscopy for TB diagnosis exist: culture of sputum on solid media, the gold standard for TB detection, has high sensitivity and specificity and allows drug susceptibility testing, a particularly important advantage in the light of the rapid spread of multidrug-resistant TB (MDR-TB) and XDR-TB. MDR-TB is defined as strains of *Mycobacterium tuberculosis*, resistant to both isoniazide (INH) and rifampicin (RIF), the two most potent antibiotics used to treat the disease (WHO 2004). Cultures, however, can take up to two months before a proper diagnosis can be provided to the patient (Narayanan et al. 2003). Although, *M. tuberculosis* can be detected in less than four weeks in a positive culture, an incubation of six to eight weeks is often required before a culture can be classified as negative.

To address that issue, culture systems based on liquid media, such as BACTEC MGIT 960 (Becton Dickinson) and BacT/ALERT (Organon Teknika), have been developed (Brodie and Schluger 2005). Such diagnostic tools have a shorter median time to culture positivity (13 days for MGIT 960) (Moore et al. 2006), but the median time to susceptibility test results is still relatively long (22 days for MGIT 960) (Moore et al. 2006). Liquid cultures present several other drawbacks: culture contaminations are systematically reported in studies, culture facilities and reagents are still expensive for implementation and scale-up in developing countries, and a recent meta-analysis of BACTEC MGIT 960 showed wide variations in the diagnostic performances (Cruciani et al. 2004).

Bacteriophage-based tests have been presented as promising candidates for the inexpensive and rapid detection of *M. tuberculosis* in the clinical specimen (Traore et al. 2007). Cost of reagents is estimated at less than \$5 and results are usually obtained in 48 hours. However, a recent meta-analysis stressed the high variation in sensitivity (ranging from 21 to 88%) of the phage-based assays (Kalantri et al. 2005). These tests require further research to enhance their sensitivity before they can be successfully implemented.

Another liquid culture-based technique, the Microscopic-Observation Drug-Susceptibility (MODS) assay, was recently shown to demonstrate promising results directly from sputum samples (Moore et al. 2006). MODS has a high sensitivity (97.8%), a median time to culture positivity of seven days, and provides drug susceptibility results in a week. However, several drawbacks have been associated with MODS:

- it is technically more challenging than the smear microscopy;
- it requires an inverted microscope, which is not readily available in laboratories that perform diagnostic tests for TB (Palomino et al. 2007);
- laboratory technicians are unable to distinguish between TB and non-tuberculous mycobacteria (NTM), which could have a potential clinical impact in settings where NTM prevalence is high;

- Finally, a critical safety issue – most laboratories where smear microscopy is performed do not have functioning bio-safety hoods that are necessary to safely examine live cultures. The liquid cultures required for MODS are in plates that must be transported from an incubator to a microscope. This carries risk of spillage and exposure to TB (and possibly MDR-TB or XDR-TB) (Moore et al. 2006).

In the recent years, rapid and accurate molecular diagnostic tests have been developed. Not only can these tools detect TB and provide information on drug sensitivity, they also give results within hours, do not cross-react with NTMs and allow several samples (up to 96) to be analysed simultaneously by a single technician. Rapid molecular tests are based on indirect detection of the TB bacilli rather than their visualisation under the microscope. Mycobacterial DNA or RNA, extracted directly from sputum or from the culture, are amplified and subsequently detected (for a review see Brodie and Schluger 2005). Line Probe Assays (LiPA), for instance, rely on amplification by polymerase chain reaction (PCR) of mycobacterial DNA present in the clinical specimen³ (De Beenhouwer et al. 1995). This technique is capable of detecting as few as 10–50 tubercle bacilli – 1000 times fewer than microscopy. Therefore, LiPA demonstrates a much higher sensitivity than detection by sputum smear (Brodie and Schluger 2005). INNO-LiPA Rif.TB (Innogenetics, Belgium), one such line probe assay,⁴ amplifies one region of the *rpoB* gene, where mutations causing RIF resistance occur, and detects the presence of *M. tuberculosis* complex and RIF resistance at a sensitivity of 92% and 96% respectively (Rossau et al. 1997). In the high incidence countries, RIF resistance serves as a surrogate marker for MDR-TB, as 90% of RIF isolates are also INH resistant (Musser 1995, Traore et al. 2000, Traore et al. 2006).

A comparison of the two commercially available LiPA demonstrated similar levels of high sensitivity and specificity for both tests, but a meta-analysis reviewing INNO-LiPA highlighted the slightly lower sensitivity (ranging from 80 to 100%) of that test when used directly on clinical specimen (Morgan et al. 2005). This meta-analysis, however, only reviewed studies that analysed limited numbers of clinical samples. A recent study on over 400 sputum samples showed that INNO-LiPA provided high sensitivity and specificity for diagnosis of TB, and there was no significant difference in detecting *M. tuberculosis* and its resistance to RIF when applied to smear positive or smear negative specimen (Traore et al. 2006). As a result, authors stressed the value of the test to rapidly adjust patient treatment and avoid risk of amplification of drug resistance. In addition, the improvement in the speed of detection leads to a reduced time to diagnosis and thus, allows for more rapid pharmacologic intervention.

Tests based on the LiPA platform, and PCR-based tests more generally, present a series of drawbacks that currently limit their implementation in the developing countries:

- PCR is technically more challenging than smear microscopy, and potentially time-consuming unless conducted in laboratories with high turnover;
- they require a PCR machine, which is not readily available;
- the current price of commercially available LiPA tests;
- in laboratories with inappropriate settings (where pre and post PCR rooms are not physically separated), there is a risk of cross-contamination when PCR is performed several times with the same target.

However, in a recent study performed in Rwanda (Quezada et al. 2007), our team demonstrated that INNO-LiPA could be implemented in a resource-constrained setting: PCR and LiPA techniques were successfully taught in only two days to microbiologists with no prior experience in molecular biology. Samples were successfully analysed for both the presence of TB and RIF resistance (up to 95% homology with results obtained by a team in Belgium), and no cross-contamination was observed during the study.

The impact of implementing LiPA into the normal functioning of a national TB programme has yet to be illustrated. However, in Latvia, a country with epidemic MDR-TB, LiPAs were successfully introduced in the national laboratory and improved the rapid detection of MDR-TB to 1–5 days, compared to 12–47 days for BACTEC (Skenders et al. 2005). LiPAs reduce the time an MDR-TB patient spends at a TB ward and thus, decrease the duration of infectiousness.

Despite the availability of LiPA tests, WHO has not yet recommended selective use of these diagnostic tools, effectively keeping microscopy as the sole cornerstone of DOTS. Two reasons are often put forward:

First, the low-cost diagnostic tool is well suited for resource-limited settings (Brodie and Schluger 2005). Microscopes do not require electricity and sputum smear staining protocols are relatively easy for technicians to learn.

Second, the cases that microscopy detects – smear positive pulmonary TB – are the most infectious, causing on an average 10–15 new infections per year (Dye et al. 2005). They are, therefore, a primary target in order to control TB. NTPs implementing DOTS are expected to detect at least 70% of all estimated smear positive cases, and successfully treat 85% of the patients diagnosed⁵ (StopTB-Partnership 2006).

The use of microscopy as the sole diagnostic tool for TB ensures the disease's continuous spread:

- It neglects smear negative and extrapulmonary TB (EPTB) cases, which account for roughly half of all TB cases (Dye 2006). Although, smear positive cases are responsible for most transmission of TB, recent studies indicate that smear negative cases contribute much more to ongoing transmission than previously believed (Behr et al. 1999). Moreover, HIV co-infected patients are more likely to produce smear negative sputum samples or develop EPTB, making this deficiency of microscopy a particularly severe liability in areas with high HIV prevalence.

- It overlooks the growing threats of MDR-TB and XDR-TB since microscopy provides no information on drug susceptibility (WHO 2004). While a six-month regimen to treat susceptible TB costs, on average, \$14–\$18 per patient (Global Drug Facility 2007), drug-resistant TB, if treatable, can take up to two years and costs several thousand dollars (WHO 2004). Thus, when microscopy misses a case of drug-resistant TB, and allows it to spread further, the long-term financial toll is far higher. In essence, a missed case of susceptible TB, which goes on to infect perhaps five other people, might have a total treatment cost of \$70; a similar missed drug-resistant case will have a cost orders of magnitude greater. Hence, in terms of TB control, there is a significant cost incurred by continuing to restrict diagnosis to susceptible smear positive cases alone.

Microscopy cannot detect smear negative cases, performs poorly with EPTB, and does not detect drug resistance. Consequently, in areas with high HIV or MDR-TB and XDR-TB prevalence rates, a control strategy based on microscopy is clearly unsatisfactory. For many countries still struggling to reach the 70–85 targets, keeping a microscopy-based approach to target smear positive cases is justified; however, other countries, such as India, are ready to roll out expanded control and elimination programmes taking advantage of powerful new diagnostic tools.

TB in India

India is in a unique position with respect to the global TB epidemic. With two million new TB cases annually, India accounts for one-third of the disease's global burden (WHO 2006d). One person dies from TB in India every minute – more than 1000 a day and 450,000 every year (Khatri and Frieden 2002a). However, India has also shaped the face of TB control worldwide: pioneering studies in India demonstrated the effectiveness of ambulatory treatment of TB (Tuberculosis Chemotherapy Centre 1959), the necessity and feasibility of direct observation of treatment (Fox 1962), the efficacy of intermittent treatment with anti-TB drugs (Tuberculosis Chemotherapy Centre 1964), and the feasibility of case detection by the sputum-smear microscopy in primary health institutions (Banerji and Andersen 1963). India has therefore, a record of setting up groundbreaking TB control projects that have the potential to define new international guidelines.

Recognising the devastating socio-economic impact of TB, the government of India launched in 1997 an ambitious Revised National Tuberculosis Control Programme (RNTCP) based on DOTS (Chauhan and Tonsing 2005). Despite criticism over the slow speed of implementation (Sharma 2003, Sharma 2004), it is the world's fastest growing DOTS programme in terms of population coverage and patients treated (WHO 2006d). This rapid expansion was promoted by several key factors (Khatri and Frieden 2002b): (1) strengthening infrastructures in the rural areas; (2) supporting the infrastructure required in the urban areas;

(3) ensuring technical excellence; (4) providing support for staff; and (5) continuous supervision. New policies introduced since the inception of the RNTCP have thus been successful in improving access to care, the quality of diagnosis and the likelihood of successful treatment (Khatri and Frieden 2002b). In 2004, the RNTCP reached the 70–85 targets (TBC-India 2005) for sputum smear positive pulmonary cases, unlike other high-burden countries in the South East Asia and Western Pacific regions, such as China and Indonesia, who reported DOTS case detection rates of 63 and 53% respectively (WHO 2006d). In 2005, the RNTCP also reached its goal of 100% DOTS coverage (TBC-India 2005), whereas China completed 96% (WHO 2006d).

India is therefore, ready to address the two greatest challenges to its national TB control programme: MDR-TB and TB/HIV co-infection (StopTB-Partnership 2006). These challenges were highlighted by several studies as hindering further DOTS expansion (Khatri and Frieden 2002b, Dye et al. 2005). We argue that the promotion of an inadequate diagnostic tool, microscopy, will not tackle the current trends of rising MDR-TB and TB/HIV co-infection rates in India, and that the RNTCP is well positioned to upgrade its diagnostic infrastructure and incorporate rapid molecular tools in order to address both challenges. Improved TB diagnostics can initially be rolled out only at top tier reference laboratories and hospitals, as demonstrated by the successful introduction of HIV diagnostics, and could save up to 105,000 lives annually (Keeler et al. 2006). Large urban tertiary care facilities already serve as models for operational research relative to RNTCP guidelines, and results are excellent (Tahir et al. 2006). With this strong research base, and a history of shaping TB control policies, India can successfully develop and monitor innovative TB programmes.

MDR-TB in India

Drug resistance is a growing threat in India: close to 2.5% of new cases and 4% of all TB cases are MDR-TB (WHO 2004, WHO 2006d, Zignol et al. 2006), and it ranges from eight to 67% in unsuccessfully treated cases (Chadha 2005). The infectiousness of MDR-TB cases, relative to susceptible smear positive TB, is still unclear (Cohen et al. 2003). Therefore, a cautious control strategy should consider MDR-TB cases as infectious as susceptible TB.

Because microscopy alone does not detect drug resistance, MDR-TB cases in India are not placed on tailored drug treatment regimens soon enough. A person presenting with MDR-TB will be initially placed on the same treatment as all other TB cases⁶ (see Figure 1). Months will pass before learning that the treatment is failing.⁷ Frequently, the patient will be wrongly blamed for non-compliance. The related delays in diagnosis and proper treatment have disastrous consequences on MDR-TB's spread. Hence, the RNTCP should consider complementing microscopy diagnosis with existing rapid tools for the detection of resistant strains at high sensitivity levels.

In order to address MDR-TB, the country is in the process of putting together treatment programmes, and several provinces have applied to the Green Light

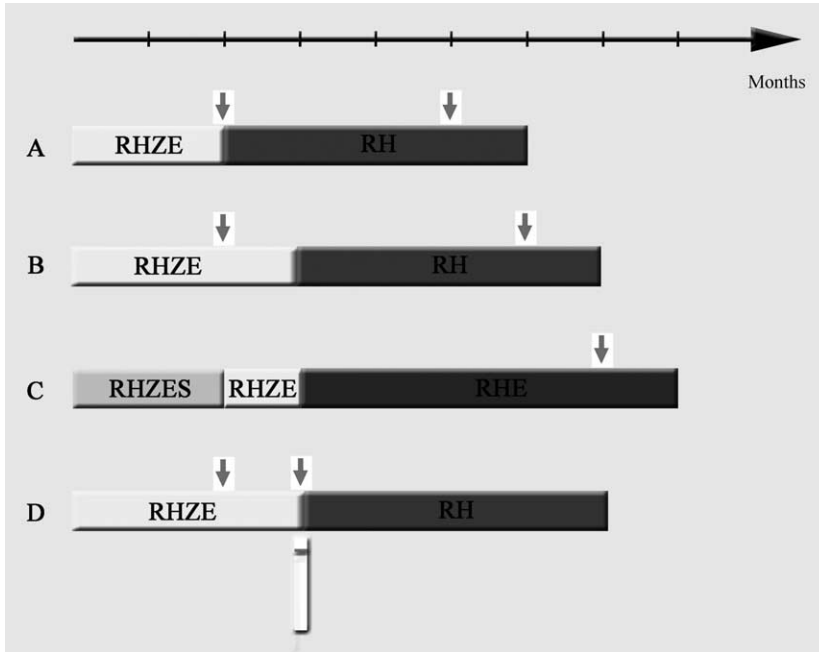


Figure 1. Tuberculosis treatment regimen. [A] Category I regimen. The treatment recommended by WHO guidelines for new cases of smear-positive tuberculosis is a six-month therapy divided in two phases: intensive (in yellow) associating Isoniazid-H, Rifampicin-R, Pyrazinamide-Z and Ethambutol-E every day for two months and continuation (in blue) which is a combination of H and R three times weekly for four months. Treatment is monitored by sputum analysis (red arrows) at the end of the second month (to monitor start of continuation phase) and at the end of the fifth month (to determine treatment success). [B] If the patient's sputum is still positive at the end of the second month, intensive phase is prolonged by one month. Successful treatment is assessed at the end of the sixth month by another sputum analysis. [C] In the event a patient's sputum is still positive at the end of a Category I regimen, or if it has become positive again, patients are subjected to Category II re-treatment regimen, which is a combination of the same antibiotics used during Category I, plus streptomycin-S. Patients infected with MDR-TB strains are unlikely to respond to this lengthy treatment. [D] To avoid late detection of MDR-TB that could be fatal to patients and detrimental to contacts, patients who are still positive by sputum smear at the end of the third month of intensive phase (Category I) should be subjected to rapid testing by Line Probe Assay.

Committee (Chaudhury and Thatte 2003). The Indian NTP also recently initiated a five-year pilot project to implement DOTS-Plus at sites throughout the country. DOTS-Plus builds upon the traditional microscopy-based DOTS to include diagnosis of MDR-TB patients and treatment using second-line drugs. Unfortunately, India relies on the solid media culture for detection of multidrug resistance—thereby missing a crucial opportunity to create a far more efficient and effective programme by not incorporating rapid diagnostics such as LiPA in the diagnostic algorithm. Several studies in India have already shown that PCR is an excellent diagnostic tool for TB (Jatana et al. 2000, Singh and Seth 2002), and that LiPAs constitute an important molecular method for the control of the disease (Sharma et al. 2003, Srivastava et al. 2004). Moreover, LiPAs are already

available on the Indian market. Therefore, we recommend that LiPA should become the new platform for detection of MDR-TB in India. We cite several reasons for the same:

First, LiPAs provide quick and reliable diagnosis within hours, as opposed to several weeks for culture (Brodie and Schluger 2005). Patients can therefore, be put on treatments tailored to their infection within days. Disease transmission and case fatality rates, especially of MDR-TB, would be significantly reduced. Furthermore, patients would no longer undergo multiple full length treatments, which are shown to considerably reduce compliance (Park et al. 1996). Additionally, results are much easier to read using LiPA, as it relies on visualising the presence of the bands on a nitrocellulose strip, much like a pregnancy test (see Figure 2).

Second, LiPAs, unlike cultures, do not require the presence of live *M. tuberculosis* bacteria in the sample. Unlike RNA-based rapid diagnostic tests, LiPAs are DNA-based and, therefore, more robust. These features simplify the handling of specimens, which do not have to be refrigerated and can spend several days in transport to the closest reference laboratory.

Third, PCR-based diagnosis of TB has been shown to be more cost-effective than microscopy, even in resource constrained settings (van Cleeff et al. 2005). Prices of PCR machines⁸ are currently comparable to those of microscopes. Due to recent patent losses by the Swiss pharmaceutical company Roche, prices around the PCR platform are expected to decrease further in the coming years (Unknown 2005). This should translate into lower entry costs for manufacturers of PCR based diagnostics. The expansion of a LiPA based DOTS-Plus is contingent on the pricing: prices of LiPAs are expected to decrease significantly in the coming years, as the Foundation for Innovative New Diagnostics (FIND) recently signed an agreement with one of the LiPA manufacturers in Europe to provide the diagnostic tools at a preferential rate for developing countries (FIND 2006).

Fourth, once in place, the PCR infrastructure should not be limited to TB diagnosis. Several procedures, ranging from the determination of HIV viral loads to the diagnosis of Hepatitis C Virus infection (Yang and Rothman 2004), also involve PCR. PCR is an expandable technology which could be critical in the fight against a wide array of infectious diseases.

Finally, rapid detection of MDR-TB helps to break the cycle of transmission and protect against an amplifier effect in which MDR-TB patients receive traditional treatment while their MDR bacilli multiply. As new antibiotics currently in the pipeline are brought to the market over the course of the next ten years, profiling each patient's infection will be critical to prevent natural selection of resistant strains of *M. tuberculosis*. This strategy will significantly reduce the chances of treatment failure (Furin et al. 2000). After waiting decades for the development of new drugs against TB, the Indian NTP must ensure the longevity of those new antibiotics by promoting both rapid drug susceptibility testing and early treatment of MDR-TB.

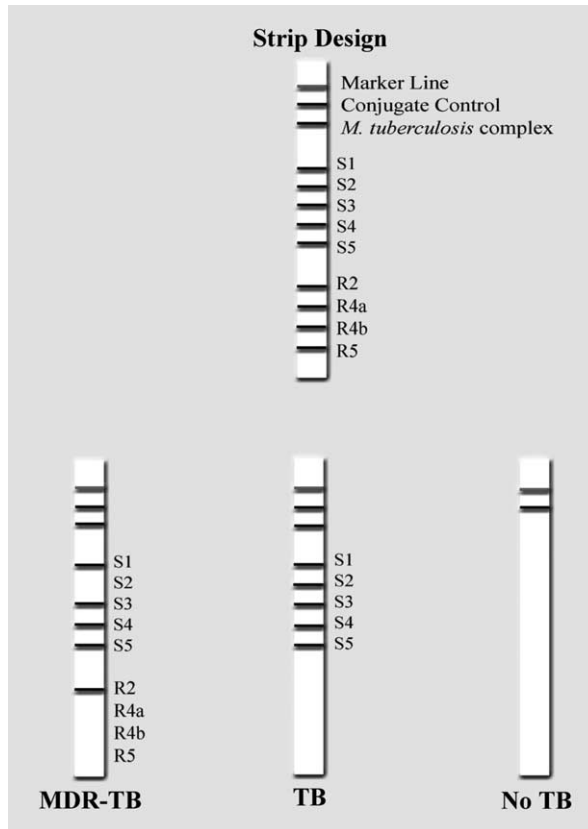


Figure 2. INNO-LiPA Rif.TB strip. The LiPA strip simultaneously detects the *M. tuberculosis* complex and the presence of mutations in the *rpoB* gene associated with resistance to RIF, which is considered a marker for multidrug resistant strains (Strip Design). The conjugate control line determines whether the diagnosis has been properly carried out. If it is the only band that appears at the end of a diagnosis procedure, TB can be ruled out for the patient (No TB). The presence of *M. tuberculosis* in a sample is detected with the *M. tuberculosis* complex-specific probe included on the nitrocellulose strip. Five partially overlapping probes (S1 through S5) of 19 to 23 bases that exclusively hybridise to wild-type *rpoB* sequence are included. If those bands appear at the end of a diagnostic procedure, the patient has active TB, but is not infected with an MDR-TB strain (TB). The reactivity of an amplified fragment with one or more of the five wild type probes (S1–S5) will be prevented if a mutation is present in one or more of the probe region. Four additional probes, R2, R4a, R4b and R5 are expected to hybridise to mutant sequences of the four most commonly observed mutations. If one of those bands appears at the end of a diagnostic procedure and/or if one of the five S bands disappears, then the patient is infected with a resistant strain (MDR-TB).

A recent study from India recommends drug susceptibility testing and treatment modifications solely for patients who do not respond to Category II therapy at the end of the third month (Santha et al. 2006). This strategy implies that drug-resistant cases will go undetected for nine months (see Figure 1). If the culture is employed, the aforementioned patients will not be put on an adequate treatment for an additional two months. As a result, MDR-TB patients will infect contacts for approximately a year, while remaining at high risk of death themselves.

Several studies have reported successful MDR-TB treatments in India (Sharma et al. 1996, Singla et al. 2001). Although, MDR-TB is expensive to treat, WHO's Green Light Committee price reduction of second line drugs has made therapy more affordable (Kim et al. 2003). A study from Peru has shown that the cost per Disability-Adjusted Life Year (DALY)⁹ saved by undergoing MDR-TB treatment is \$211, with an average treatment cost of \$2 381 (Suarez et al. 2002). For comparison, antiretroviral therapy costs between \$260 and \$530 per DALY gained (Currie et al. 2005). Since, the cost per DALY saved is lower than India's per capita annual gross domestic product, international lending agencies are likely to deem infrastructure upgrades a reasonable investment (Turett et al. 1995). Opportunities to address MDR-TB in the developing countries are now possible through the Global Fund to Fight AIDS, TB and Malaria (Mukherjee et al. 2004), which can provide resources directed specifically towards the diagnosis and treatment of MDR-TB cases. This will hopefully shift the debate to a new level: the ethical issues raised by not treating MDR-TB cases (Mukherjee et al. 2004, Yong Kim and Shakow 2005).

As suggested by others (Gupta et al. 2001a, Gupta et al. 2001b), the MDR-TB threat in India requires a prompt response. Whenever possible, we recommend administering LiPA for all the patients failing intensive phase, as determined by a positive smear at the end of the third month. This should identify the population of resistant cases and separate them from those patients with susceptible TB that are still positive at three months, and who can be directed towards the standard continuation phase. MDR-TB patients will therefore go undetected for only three months (see Figure 1) instead of 11 (Santha et al. 2006), which will significantly reduce transmission and mortality rates. Patients who have previously been on anti-TB therapy (both treatment failures and re-treatment cases) should also systematically undergo LiPA to rule out MDR-TB, before initiating a Category II re-treatment regimen. Finally, all contacts of MDR-TB cases should be screened for active TB, and LiPA administered for all the confirmed cases.

Tuberculosis (TB)/HIV co-infection

With five million Indians currently living with HIV/AIDS (Godbole and Mehendale 2005), India has a significant AIDS epidemic (Rao et al. 2004, Mahal and Rao 2005), further complicating the battle against TB. An expanded HIV epidemic would have severe consequences for the burden of TB. TB is the most common opportunistic disease among HIV-infected people worldwide (Arora and Kumar 1999, Kothari and Goyal 2001). As patients infected with HIV develop AIDS, their risk of developing TB increases (Williams and Dye 2003). HIV co-infection complicates the treatment of TB patients under antiretroviral therapy as drug interactions occur with RIF, which must therefore be replaced by an analogue such as rifabutin (Finch et al. 2002). Patients infected with HIV have also been shown to be at higher risk of acquiring MDR-TB (Busillo et al. 1992, Edlin et al. 1992, Frieden et al. 1993, Faustini et al. 2006). In latent cases, the probability of progression into active TB increases, from 10% over a lifetime for

HIV negative patients, to 10% a year for HIV infected patients (Alland et al. 1994). Partly due to these increased reactivation rates, over 5% of TB cases, aged 15–49, in India are also HIV positive (Chadha 2005, WHO 2006d). Mathematical models have shown that in order to successfully control TB in India, the RNTCP must address HIV (Williams et al. 2005).

HIV further poses a threat to the management of TB because co-infected patients are more likely to produce smear negative sputum samples (Wood et al. 2000, Corbett et al. 2003), or develop EPTB (Ong et al. 2004, Yang et al. 2004). Since microscopy cannot detect smear negative cases, and performs poorly with EPTB (Honore-Bouakline et al. 2003, Chakravorty et al. 2005), radiography is usually recommended. The results, however, are unreliable (Harries et al. 1998).

The WHO guidelines, based on the recent algorithms (WHO 2006e), advise clinicians to initiate TB treatment for smear negative cases in patients of positive or unknown HIV status if the following conditions are met.

At least two sputum smears are negative by microscopy detection, and:

- I. A chest radiograph is compatible with active TB,
Or
- II. A culture is positive for *M. tuberculosis*,
Or
- III. There is no improvement after a course of non-TB-specific antibiotics.

These guidelines are ill-defined because they rely on the interpretation of a chest radiograph, which is particularly difficult to analyse in HIV patients co-infected with TB¹⁰ (Colebunders and Bastian 2000, Siddiqi et al. 2003). There have also been questions around the efficiency of the antibiotic course for diagnosis of TB, as response to the antibiotics does not exclude TB in HIV prevalent settings (Fourie and Weyer 2000, Siddiqi et al. 2003). Clinicians caring for HIV-infected patients, with suspected sputum smear negative TB and EPTB, have to weigh the possibility of active disease against the cost, inconvenience, and potential cross-reactivity, associated with a full course of anti-TB treatment. This results in significant delays before initiation of therapy. Late diagnosis leads to increased infectiousness because patients have more time to become smear positive and develop pulmonary cavities. Delays in clinical suspicion and proof of diagnosis are the most common causes of preventable morbidity and mortality. This re-inforces the need for a rapid and sensitive test for smear negative and EPTB (Kwara et al. 2004). If the high mortality figures associated with smear negative TB in HIV-infected adults are to be reversed, then diagnostic strategies must be improved.

In order to address diagnostic issues associated with HIV co-infection in India, we recommend administering LiPA to all cases where the disease is clinically suspected (persistent cough, chest pain, weight loss, fever and night sweats) but microscopy is negative (Tiwari et al. 2003). Studies have confirmed the high sensitivity and specificity of LiPA for detection of smear negative (Cirillo et al.

2004, Traore et al. 2006) as well as EPTB (Gamboa et al. 1998). Consequently, clinicians would have a powerful confirmatory test offering several key benefits:

1. smear negative cases would be diagnosed within a day directly from sputum, suppressing the need for chest radiograph, and limiting the risk of having patients not return to the clinic after a course of non-TB-specific antibiotics;
2. EPTB cases would be accurately diagnosed from the clinical samples, such as spinal fluids, significantly reducing mortality rates; and
3. technicians could analyse up to 96 samples at a time per PCR machine, thus, effectively alleviating the acute human capacity constraint faced by the NTP in India (Chauhan 2003).

Conclusion

The WHO should reconsider the universal application of DOTS by recommending the selective use of new molecular diagnostics in certain settings for detection of MDR-TB and TB in HIV co-infected patients. India's RNTCP is far more advanced than its counterparts in many African countries, and a suitable candidate for diagnostic infrastructure upgrades to incorporate detection of MDR-TB, EPTB, and smear negative cases at the district level. India is ready to roll out expanded control and elimination strategies by complementing sputum smear microscopy with LiPAs.

With the emergence of resistant strains worldwide, a toolkit, which appropriately addresses the TB and MDR-TB threat, must now be put to use. LiPA offers precisely the instrument desperately required and uses technology widely available, particularly in India. The savings will be enormous and, most importantly, it holds open the promise of ensuring immediately effective treatment of TB rather than the current wait-and-see approach.

When HIV diagnostics were first developed, there was a critical mobilisation to promote their use in resource-poor settings regardless of their price. By the same token, the treatment of HIV, in spite of its substantial costs in poor countries, continues to move forward as it should. It is now time for TB to be given similar attention – financially, politically and technologically.

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Notes

- ¹ Bacille de Calmette et Guérin.
- ² Directly Observed Therapy – Short Course (DOTS).
- ³ Subsequent specific hybridisation of PCR amplicons on the nitrocellulose strips detects the presence of bacteria in the specimen under scrutiny.
- ⁴ Two LiPAs are currently available on the market: GenoType® MTBDR (Hain Lifescience GmBH) and INNO-LiPA Rif.TB (Innogenetics).
- ⁵ Subsequently referred to as ‘70–85 targets’.
- ⁶ Standard WHO-recommended treatment for TB, or Category I, is divided into two phases: two months of isoniazid, RIF, ethambutol and pyrazinamide taken daily (intensive phase), followed by 4 months of isoniazid and RIF taken three times a week (continuation phase).
- ⁷ Treatment failure cases undergo standard re-treatment regimen, or Category II, consisting of the same antibiotics used for Category I, plus one additional, streptomycin.
- ⁸ Excluding real-time PCR.
- ⁹ Disability-Adjusted Life Year.
- ¹⁰ Immuno-compromised patients are more likely to have chest radiographs without the evidence of cavitation.

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