

HATiP

HIV & AIDS Treatment in Practice

Issue 164 | 27 August 2010



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Towards more compassionate and effective care for drug resistant TB: improving diagnosis and case detection

By Theo Smart

This edition is kindly supported by the Stop TB department of the World Health Organization and the Diana Princess of Wales Memorial Fund.

It forms the second of a three-part clinical review series on improving care for people with HIV who have multi-drug resistant tuberculosis. The first part ([Towards more compassionate care for people with drug-resistant TB](#)) was published in July 2010.

We would like to thank Dr Karin Weyer (WHO) for her comments and advice on this edition. Lance Sherriff contributed additional reporting from the Second South African TB Conference.

Key points

- This article is primarily intended for microbiologists and TB specialists, advocates and those involved in the planning of laboratory services who wish to inform themselves in greater detail about recent developments in the diagnosis of drug-resistant TB and service standards in TB diagnostics.
- Suspected TB cases need to be confirmed rapidly, especially where there is a high risk or suspicion of drug-resistant TB. Current waiting times, imposed by diagnostic inadequacies and poor funding, are unacceptable and contribute to the spread of MDR-TB and high rates of mortality in people with drug resistance.
- At a minimum, countries should be able to diagnose isoniazid and rifampicin resistance, WHO recommends.
- Screening for drug resistance is recommended for all previously treated cases, for people failing their current TB regimen, for contacts of people with M/XDR-TB, for all people with HIV, and for all new TB cases in settings with a high rate of M/XDR-TB transmission. Active case-finding is likely to reduce transmission and mortality.
- All diagnostic systems have their strengths and weaknesses. WHO is currently considering whether the evidence to support roll-out of the GeneXpert system is strong enough. This system has shown good results in demonstration studies and is easy to use even in district hospital settings without advanced laboratories.
- GeneXpert and other commercial systems may be expensive for countries that are not receiving external support to upgrade their laboratory systems. Non-commercial alternatives exist, which include microscopic-observation drug susceptibility assay (MODS). This system has shown some promise in HIV-positive people with smear-negative TB. MODS is not suitable for decentralised use but may be an interim solution for MDR diagnosis.

- Better MDR diagnosis also requires improvements in sample transport, staff training, quality control and prompt communication of results.

Improving diagnosis and case detection

Failure to prioritise the treatment of people with multi- or extensively drug resistant TB (M/XDR-TB) “no longer makes sense... given their high mortality and the urgent need to prevent the spread of these deadliest TB strains,” according to the 2010 WHO guidelines on the Treatment of Tuberculosis (TB).¹

TB control activities had traditionally stressed treating new cases with sputum smear positive pulmonary TB (which was once called category I TB) and placed much less emphasis on ‘retreatment’ or ‘chronic’, smear-negative and drug-resistant TB cases (previously categories II-IV respectively). But as the new WHO guidelines note, this is inconsistent with the current Stop TB Strategy that stresses the delivery of universal access for *all* people with TB to high quality, patient-centred care from diagnosis through treatment completion (as mandated by the Patients’ Charter for TB Care).

But to reduce the suffering and improve survival of people with drug resistant TB (DR TB) — and prevent further transmission of the illness — most health systems (including both TB and HIV programmes) will need to revise strategies and address performance in several areas all at once.

Rather than relying on passive case detection, potential cases of drug resistant TB will have to be detected much sooner, through routine assessments of treatment failures, defaulters and relapses, intensified case finding, screening campaigns and contact tracing. However, suspected cases cannot be confirmed quickly without scaling up laboratory capacity to do reliable and timely drug sensitivity testing (DST), preferably using a rapid test, such as a line probe assay (see later in the article) to quickly flag cases with resistance and then culture-based DST methods to better characterise the resistance (and optimise the treatment regimen).

It can be crucial to get someone on an effective regimen as quickly as possible, and in some cases (treatment failures and contacts of MDR-TB cases) empiric treatment for drug-resistant TB may be necessary. But that won’t be possible if programmes haven’t implemented reliable mechanisms to procure all the medications necessary to effectively treat M/XDR-TB — including the drugs needed to help manage the side effects and common clinical complaints among people with M/XDR-TB.

TB programmes also need to guarantee that HIV screening happens concurrently (in anyone suspected of having TB) and that antiretroviral treatment is begun as soon as possible after TB treatment has been initiated. Models for the local delivery of M/XDR-TB care and treatment should be reviewed to make certain that people are put onto appropriate treatment as soon as possible after diagnosis, and stay in care — without losing people in a referral process — and that there are adequate systems in place for case management to support adherence and to monitor for side effects and other problems, including financial and psychosocial impact of the illness upon the client and his or her family.

This HATIP addresses the laboratory infrastructure that will be needed to improve case detection of M/XDR-TB.

M/XDR-TB diagnosis

TB symptom screening and the basics of TB diagnosis have been discussed in other issues of HATIP, including the challenges of diagnosis in a person with HIV. The likelihood of getting an accurate diagnosis of drug-resistant TB is even poorer globally and

represents a weak link in the effort to provide more compassionate and effective care for people with M/XDR-TB.

The process of diagnosing cases involves first identifying people with TB at risk of drug resistance (case detection — see later in the piece) and then sending their specimens to the laboratory for DST. However, the costs and laboratory capacity to perform culture and DST varies dramatically from country to country, and are important considerations in the choice of case detection strategies (i.e., which cases to prioritise and how widely to cast the net). Moreover, over the last several years, the TB diagnostics field has been in a state of flux, so a review of DST may first be in order.

At a bare minimum, the WHO recommends that countries implementing a DR-TB programme have to be able to perform DST for isoniazid and rifampicin.² DST can be either culture-based, measuring mycobacterial growth (or metabolism with some experimental assays) in the presence of drug-containing media (to assess phenotypic resistance), or molecular-based (polymerase chain reaction (PCR)/nucleic acid amplification tests that look for specific mutations associated with resistance (genotypic resistance).

But, as the case of Lesotho — which recently has scaled up laboratory capacity to do both liquid culture and molecular DST (see below) — illustrates, the usual place to start is by first establishing culture-based DST at centralised reference laboratories, though a number of DST platforms now being evaluated (both commercial and non-commercial) could be more suitable for more decentralised use.

Conventional drug susceptibility testing (DST)

Although slow, **conventional DST on solid media** (egg-based such as Löwenstein-Jensen (LJ) culture medium or agar-based) continues to be used worldwide because it is inexpensive and the methodology is standardised for a wider variety of TB drugs, including some (though not all) of the second-line drugs.³

After culturing (which can take up to two months), resistance can be detected depending upon whether there is growth when the cultured strain is placed in drug-containing media. There are three solid culture DST methods the proportion method, the resistance ratio method, and the absolute concentration method — which each achieve similar results — but the proportion method is the most widely used and best characterised for second line DST.⁴

However, even though conventional DST is considered the gold standard, there are still limits when it comes to detecting resistance to second-line drugs. For instance, the reproducibility and reliability of DST results for the thioamides, para-aminosalicylic acid, and serine analogues are poor — which, of course, makes it more difficult to optimise treatment regimens.

“DST is technically complex,” said Dr Karin Weyer (now at WHO) during a presentation at the World Union Lung Health Conference a few years ago.⁵ “Drug powders are often unstable in *in vitro* testing and critical drug concentrations defining resistance are often close to drug MIC and/or attainable serum levels, so the results are therefore inconsistent.”

Furthermore, she noted that the degree of cross-resistance within classes of drugs, such as aminoglycosides and polypeptides is poorly characterised (and this now appears to be true for the newer fluoroquinolones as well)⁶ and second line DST findings do not always correlate with a clinical response. Recently though, WHO conducted a systematic review of the published literature to produce a tentative consensus on what is known in DST for

second-line drugs in the *Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs*.⁷

Nevertheless, getting results from conventional DST can take from three to four months — too long for people with advanced HIV disease and drug resistant TB to wait for effective second line treatment. While, the capacity to perform conventional DST remains necessary for confirmation, complex cases and for surveillance purposes, programmes will need to introduce other DST methodology for patient management purposes.⁸

Well-resourced countries may be able to adopt multiple DST platforms, but many programmes in resource limited settings face a difficult choice between different technically sophisticated but expensive commercial platforms that have been extensively field tested by the manufacturers as well the Foundation for Innovative Diagnostics (FIND). In addition, there are also simpler low cost methods which may actually be more practical in some resource-limited settings, but which do not have a commercial backer and thus have not been as extensively studied.

Commercial DST platforms

Liquid culture

methods, such as the mycobacteria growth indicator tube (MGIT) which can be performed manually or on automated systems (such as Becton Dickinson's BACTEC MGIT 960 system) are the standard in developed countries and shorten the turnaround time to results considerably compared to conventional DST.

Unfortunately, the technically advanced equipment and consumables are quite expensive to both purchase and maintain.

Furthermore, contamination rates in the field can be high unless strict quality control measures are put in place.

Rapid speciation with tests such as the Capilia TB assay is also necessary, since non-tuberculosis mycobacteria (NTM) also grows quite well in liquid culture. However, a recent study in Zambia and South Africa reported that the Capilia TB assay is both rapid and simple to perform and only added US \$1.46 and \$1.84 when added to the cost of performing liquid and solid culture processes, respectively.⁹

The large automated systems have a high throughput, and are clearly intended for centralised reference laboratories. Even so, once the specimens have been received, liquid culture systems can confirm TB in a week or two, (and can take up to 42 days to be certain of a negative result). Once cultured, it can take another week or so to detect first-line TB drug resistance and there is growing clinical experience using it for second-line drug resistance.^{10,11} WHO has endorsed the use of liquid culture and DST in low- and medium-income settings, provided that the required infrastructure and biosafety measures are in place, and that affordability and sustainability are ensured.¹²

A number of mycobacteriophage-based assays are in experimental development, such as the FastPlaque TB-Rif. Mycobacteriophages, which can have or be engineered to have a number of properties such as fluorescence, specifically infect mycobacterium, and TB drug resistance can be detected quickly by measuring for their replication in MTB that has been exposed to drugs. However, there were problems with contamination and indeterminate results with the earlier generation of FastPlaque, and commercial platforms for other mycobacteriophage-based assays have yet to be developed.¹³

Molecular tests

look for the genetic mutations or single nucleotide polymorphisms, through extraction and amplification of a targeted gene in a sample, that have been associated with clinically relevant drug resistance. While these mutations are fairly well characterised for rifampicin and isoniazid, mutations that cause resistance are not fully understood for many drugs. In fact, even for rifampicin and isoniazid, there are other mutations, that may be tied to a decrease or increase in phenotypic susceptibility in a small number of people with TB, that molecular tests simply aren't looking for.¹⁴

Molecular **line probe assays (LPAs)**, such as the GenoType MTBDRplus assay from Hain LifeSciences and the Inno-LiPA Rif TB assay from Innogenetics, amplify target genes in an isolate or smear positive sputum specimen that binds to DNA probes immobilised on a nitrocellulose strip. Through an enzymatic colour reaction, the resistance mutations show up as bands on the strip that can be interpreted through comparison with a template included with the assay. The current generation LPAs are sensitive and specific for rifampicin resistance, either alone or in combination with isoniazid though future generation LPAs may include probes for resistance to ethambutol and second line drugs.

The chief advantage of the LPA is that it is fast, with results in one or two days though the actual turn-around time can be longer, depending upon how specimens are delivered to the reference laboratory and how the results are sent back.¹⁵ (Also how many specimens, as the throughput is about 96 specimens per lab tech per day). Once integrated into MDR-TB screening algorithms, not only should this reduce the risk of putting someone with MDR-TB on a failing regimen, it should screen out people with drug susceptible TB and thus significantly reduce the demand on culture and DST laboratory capacity (provided clinic staff are actually sending in specimens for case detection).

LPAs require some basic PCR infrastructure and expertise, including separate rooms/work areas to prevent contamination — which may need to be built if the lab does not already have facilities for PCR. “The necessary infrastructure for performing LPA should be considered prior to implementation — a minimum of three separate rooms is recommended to minimise the risk of contamination,” wrote the authors of a recent study, who reported good experience implementing the system in Uganda.¹⁶

In most resource-limited settings, these requirements mean the test can only be introduced into centralised reference laboratories, although some countries, including South Africa, have already introduced capacity to perform LPA into reference labs in each province.

Fortunately, however, LPA is not difficult to perform with a minimal amount of training and support — in fact, in the Ugandan study, “the laboratory technologists performing the assay... had no previous experience of molecular diagnostics, and had undergone 4 days of LPA training immediately prior to starting the study,” the authors wrote. However, they also noted that there were some “initial problems with invalid results, as well as difficulty interpreting weak mutation bands [there can be shades of grey] and dealing with contamination, [so] a longer supervised period during early implementation of the technology is advisable.”¹⁷

Another advantage of using LPA is that mTB in the specimen can be killed, so the technique poses much less of a safety risk for transportation and laboratory personnel. Inactivated specimens can even be posted in the mail. One downside however is that, for smear negative specimens, culture is still necessary.

WHO has endorsed the use of validated LPAs in screening for isoniazid and rifampicin resistance, “provided that technical

expertise on molecular techniques and proper facilities are in place and sustainability is ensured.”¹⁸

Once again however the cost of the equipment is a barrier to wide scale-up of LPA in resource-limited settings, though FIND has negotiated significant price reductions with the manufacturer to obtain the equipment and reagents for LPA testing by the public health sector in high burden countries. Still, as authors of the Ugandan study point out, programmes still must pay for some consumables and reagents not provided as part of the kit, such as pipette tips and molecular grade water — and the investment in basic molecular laboratory infrastructure.

Another molecular test could potentially be installed into large clinics and district hospitals without advanced laboratory infrastructure. **Cepheid's GeneXpert MTB Rif-assay** is a self-contained system that automates sample processing on sputum and real time PCR detection, diagnosing both TB and rifampicin resistance in under 2 hours. Since it is a closed system there is no risk of contamination or need for a safety cabinet.

The test is exceedingly simple to perform, according to Sharmila Naidoo, the TB laboratory manager at Lancet Laboratories in South Africa, who described her lab's experience with the test at a session of the 2nd South African TB conference.

“The GeneXpert can be performed directly on smear-negative and smear-positive samples. You take one ml of sputum mixed with two ml of sample treatment buffer. This is mixed, incubated for 15 minutes, placed into the GeneXpert module and [voila] you have the result in one hour and 38 minutes,” she said.

In another presentation at the conference, Dr Leslie Scott also stressed how easy the GeneXpert was to use, particularly in comparison to other molecular/nucleic acid amplification tests (including the LPA).²⁰

“The Hain and LightCycler [another molecular test from Roche] required technically trained operators with at least 2 days training, but the Xpert could be performed by counsellors and nurses with about one day of training,” she said, although she pointed out the test did also have a somewhat higher invalid rate (around 7%) due to power failure, failure to show a result, cartridge error, and operator error. Nevertheless, “overall GeneXpert MDR/RIF showed best performance among nucleic acid amplification tests.”

In fact, in Dr Scott's study, the GeneXpert was 84% in agreement with culture (vs 71% agreement for the LightCycler and Hain LPA) and in contrast to the other molecular test, its sensitivity was not significantly affected by whether the specimen came from someone with HIV. The GeneXpert was 100% in agreement for cases that were smear positive and culture positive, and detected 63.9% of the cases that were culture positive but smear negative (the other molecular methods were much less sensitive in smear negative TB).

Although the GeneXpert has been optimised for performance on sputum specimens, Naidoo reported the test worked on extrapulmonary specimens as well, including urine samples, tissue, lymph node, cerebrospinal fluid and bone-marrow samples as well. “For the extra pulmonary samples there was nothing extra done, the samples were treated the same way as the sputum. Except the incubation time was slightly extended to 20 minutes instead of the fifteen-minute incubation time. In 361 extra-pulmonary samples tested, we showed a sensitivity of 93.5% and a specificity of 99%,” she said.

Naidoo acknowledged the test is quite expensive to order, approximately R600 (US \$80) — its cost will not become clear until FIND completes the demonstration projects and negotiates a reduced price for the test and its platform (which could be used for

other tests, including, HIV viral load and XDR-TB diagnosis). The throughput is also limited to 16 tests per module.

Like LPAs, the GeneXpert can be incorporated into algorithms that reduce the need for culture. "If it's MTB rifampicin sensitive we've got a result, we don't culture further," said Naidoo, "and it has alleviated the workload in the laboratory." But the hope is that they can be put at larger clinics to move MDR-TB and smear negative TB diagnosis much closer to the patient. For instance, one is being tried out at the Ubuntu clinic in Khayelitsha.

According to Dr Weyer, the GeneXpert system will be assessed by WHO this month, and rapid policy guidance will follow.

"If the evidence base for WHO recommendation is found to be adequately strong, this will revolutionise the diagnosis of TB and enable countries to move to large-scale rapid resistance testing, which then makes drug availability and the models of patient care critically important. From a laboratory perspective, even with Xpert technology widely available, conventional DST will still be required at central level for DST other than rifampicin, including second-line drugs, specifically fluoroquinolones and injectables to detect XDR," she told HATIP.

How much does this infrastructure cost to scale up?

But it's hard not to wonder what it might cost a programme to put together the commercial laboratory infrastructure that FIND has been evaluating. Fortunately, FIND has recently described the experience in Lesotho, where the combination of political will and an infusion of external funds and expertise has transformed the TB laboratory system.²¹

During a visit to the country in 2006, a team from FIND concluded that the country desperately needed to renovate the TB laboratory system. The country had launched a quality assurance programme for smear microscopy that had only been partially implemented, and essential equipment to perform DST was missing. In fact, at the time of the visit, the country had to send all the clinical specimens from MDR-TB suspects that required culture and DST out of the country at high cost to laboratories in either South Africa or the United States.

The National TB Programme (NTP) needed little convincing when FIND told them that they could help them upgrade the laboratory capacity at 'minimal cost. The only problem was that Lesotho's a very poor country, — how could it pay for all of this? So they looked to their funding partners, including WHO and Partners in Health, which was establishing a treatment programme for MDR-TB in the country. FIND provided a full-time onsite consultant and procurement of an instrument for automated TB liquid culture and DST, plus a continuous supply of reagents.

Starting in May 2007, the NTP and its partners began to develop training materials and manuals to put in place quality assurance for microscopy (an ongoing process) with external support from the supranational reference laboratory (SRL) in South Africa. At the same time, the National TB Reference Laboratory was renovated to meet biosafety requirements to perform liquid culture. Conventional TB solid culture and DST were then introduced, with external quality assurance provided by the SRL in South Africa.

During the second phase, a BACTEC MGIT960 was installed, along with the rapid Capilia TB speciation test, so that liquid culture and DST were available from December 2007. Then, starting with the construction of a clean-room facility for molecular testing in July/August 2008, the capacity to perform LPA was put in place, and training of laboratory staff took place in October 2008.

And how much did it cost? Less than US\$550 000, including \$93,000 for laboratory infrastructure upgrade, \$65,000 for TB diagnostic instruments, excluding the ones that were available but unused, \$280,000 for reagents and consumables for one year, and \$90 000 for human resources during the project.

Expandx-TB

The systemic study and field evaluation that commercial systems have received in FIND's demonstration projects has made it possible for WHO to recommend their scale-up in the field. And in order to respond to the burden of M/XDR-TB, the global MDR-TB & XDR-TB response plan, 2007–2008 estimated that over 130 advanced diagnostic centres for culture and DST would need to be developed in high-burden countries (including 5 national reference laboratories).²² That represents an enormous challenge, especially considering that the commercial options so far are complex and expensive solutions.

Recently FIND has partnered with UNITAID, the Global Laboratory Initiative and the Global Drug Facility to 'expand' access to these newer diagnostics in countries that can ill afford them on their own, with the Expandx-TB initiative.²² While FIND tries to negotiate lower prices with the suppliers and along with the Global Laboratory Initiative, provides countries with technical assistance and mentorship, the Global Drug Facility helps with procurement, while UNITAID will attempt to provide money for essential instruments reagents and supplies.

In 2009, in addition to Lesotho, the Democratic Republic of Congo, Côte d'Ivoire, Ethiopia, Myanmar and Kazakhstan benefited from the initiative, and a commitment to provide technical and financial assistance has been made to 21 other countries (including over 40 labs in India) through 2011.

Non-commercial DST alternatives

Even so, many countries with emerging drug resistance problems, must upgrade their laboratories on their own. But according to Ani et al, the authors of one recent DST study in Nigeria, "the technological expertise required, and the high cost of the non-conventional automated and molecular techniques which have shorter turnaround times (1–21 days) are some reasons that most routine laboratories are unable to perform DST for M. tuberculosis."²³

Furthermore, in today's economic climate, it is not certain how long that aid for TB laboratory scale-up and maintenance can be guaranteed.

"Once donor funding dries up, even middle-income countries may then find that only the rich can afford to pay for these tests," wrote Van Deun et al in a useful review of both commercial and non-commercial DST methods.

Increasingly, researchers and programme managers have begun to investigate less expensive alternatives to the commercial systems. Most of these are direct DST methods (performed directly with sputum or other specimens rather than indirectly from strains only after they have been cultured) also significantly shorten the time to getting a result generally to between one to three weeks and are markedly less expensive than the commercial options.

Slide DST

An old but relatively simple method, after being incubated in a liquid media (and presumably with drug) for around ten days, Ziehl-Neelsen (ZN) stained sputum smears can be checked for microcolony growth.^{24, 25} In a liquid media, MTB grows quickly and the tangle-like colonies of TB should be recognisable under a

microscope. The addition of para-nitrobenzoic acid, a specific inhibitor of *MTB* to a duplicate specimen can help differentiate *MTB* from *NTM*.

This method is similar to MODS (described below) but requires less equipment and should be safer because it involves less manipulation of the live specimens. Incubation can be done in tightly-closed, strong universal bottles that can be heated to kill the culture before opening.

Although the technique can be highly sensitive and specific, Van Deun et al state that quality assurance is a challenge, and that the accuracy of the technique is dependent upon the experience of the lab tech.²⁶ Nevertheless, in the review paper, they wrote, “slide DST is promising for early, decentralised and accessible presumptive diagnosis of RMP-resistant *M. tuberculosis*. Attached to a MDR-TB treatment centre, it allows faster management decisions, such as treatment start and failure declaration, or prompt isolation of XDR.”

Thin-layer agar (TLA) method (Microcolony method)

A team working with Médecin Sans Frontières in Medellín, Columbia have recently reported on a new technique working with thin-layer agar.²⁷ The method uses a conventional microscope to look for microcolony growth in quadrant petri plates (all containing 5 ml of 7H11 Middlebrook agar). However, while one plate contains only the growth media, one is treated with para-nitrobenzoic acid (to exclude *NTM*), one with isoniazid and one with rifampicin and then inoculated with sputum (diluted according to the acid fast bacilli load on the smear from the sample). Then the plates are sealed with tape and incubated in a CO₂ incubator. Plates were then read every 2 days over 4 weeks.

If there is growth in the plate with only growth media, but not in the plate with para-nitrobenzoic acid, TB can be diagnosed. Resistance is diagnosed based upon whether there is growth in the drug containing plates. In the Colombian study, the median time to diagnosis of TB was 10 days. The mean time to detection of resistance was 11.1 days (95%CI 6.7–15.4) for RMP and 11.25 days (95%CI 9.2–13.2) for INH and the method's sensitivity, specificity and predictive values for rifampicin and isoniazid resistance were 100%. However, it still took up to 38 days to detect resistance in smear negative samples.

Although this report is promising, Van Deun et al note that the study had a small sample size and more studies are needed. Furthermore, they suggest that the workload repeatedly reading each plate could be a disadvantage.

The nitrate reductase assay (NRA)

Another technique that is being explored doesn't even require a microscope. The nitrate reductase assay is based on the ability of living (and growing) *MTB* (but not other mycobacteria) to reduce nitrate to nitrite (the Griess reaction), which can be seen visually by the development of a dark rose to purple-rose colour after addition of the reagent.^{28, 29} The Griess reaction is a common way to speciate *MTB*. (It should be noted that there are also other colorimetric methods being evaluated including redox indicators, such as alamar blue, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and resazurin. Each produces a clearly visual and easily interpreted change of colour when there is oxygen consumption in a culture in which *MTB* is growing).³⁰

The NRA can be performed on classical LJ medium, without extra equipment, in securely closed tubes. Drug (usually rifampicin and isoniazid) can be added to the medium in the tubes that can then be inoculated using culture isolates or by direct inoculation of smear positive sputum.³¹

In 2008, a meta-analysis concluded that NRA is highly sensitive and specific for the rapid detection of rifampicin and isoniazid resistance in culture isolates, and that the data on sputum are promising.³² Since that time, more experience has been published using the NRA directly on sputum.

In Nigeria, Ani et al reported that NRA was highly sensitive and specific for rifampicin and isoniazid resistance, with results detectable within 10-14 days. However, it was not so sensitive for ethambutol and streptomycin.³³

In Sao Paulo Brazil, Shikama et al looked just for rifampicin resistance (as a marker for MDR-TB, and reported that NRA results of susceptibility were available in 15 days for 87% of the samples — however, specimens with lower AFB loads on smear microscopy could take a bit longer.³⁴

Researchers in India reported good agreement between direct NRA on sputum and conventional DST — with a turnaround time of 14 days for results in most cases.³⁵

A study in Honduras concluded that NRA may be useful for detecting XDR-TB as well, with good agreement between NRA and the BACTEC reference method, and good sensitivity and excellent specificity for isoniazid, rifampicin and ofloxacin.³⁶ Findings for kanamycin resistance were also promising though not as sensitive.

After conducting a meta-analysis of different direct DST methods, Bwanga et al of Makerere University, compared seven tests for the rapid detection of multidrug-resistant tuberculosis in Uganda including direct NRA, some of the redox indicators, MODS and the commercial assays (MGIT 960 and Hain LPA MTBDRplus).^{37, 38} NRA actually was found to be the most accurate test, with 100% sensitivity and specificity for both rifampicin and isoniazid resistance though obviously was not as rapid as the LPA (which produced results in 2 days versus 14).

Nevertheless, Bwanga et al concluded “In the study setting, NRA, MGIT 960 and Genotype MTBDRplus gave excellent detection of multidrug-resistant tuberculosis, with significantly shorter time to results compared to conventional testing.”

The microscopic-observation drug susceptibility assay (MODS)

Perhaps the best-known direct DST test, the microscopic-observation drug susceptibility assay (MODS) has its champions and its detractors.

[MODS is an 'in-house' form of liquid culture](#) — and since *MTB* grows faster in liquid media, results are available much sooner than other non-molecular DST techniques (in a median of 6 days after inoculation for smear positive samples, 7 days for smear negative samples, while a culture negative result can be determined in almost all cases by day 15).³⁹

Sputum samples are directly inoculated into 24 well plates, with or without TB drugs, placed in sealed bags and incubated with CO₂. The plates are examined each day for signs of the characteristically corded growth of *MTB* using an inverted light microscope. The presence of at least 2 colony forming units is considered a positive result.

While most studies find that the test has pretty good agreement with conventional DST, there are concerns related to the risk of contamination and safety. “Potential challenges include CO₂ supplementation and the need for meticulous technique during inoculation and plate handling to prevent (cross-) contamination. The requirement of an inverted microscope, in addition to biosafety concerns related to extensive handling of liquid cultures in 24-well

plates outside a biosafety cabinet, may restrict its use to reference laboratories,” Van Deun et al wrote.

However, there is continued interest in the technique, which should increase now that there is at least one report of MODS being successfully implemented in a high HIV incidence setting in Tugela Ferry, South Africa, as reported by Dr Sheila Bamber at the 2nd South African TB Conference.⁴⁰

The study enrolled 534 consecutive adults with TB symptoms from June 2008 through April 2009, either new cases who had received less than 48 hours of TB treatment (89%) or who were suspected to be failing TB treatment after 2 months of therapy, based on continued presence or recurrence of symptoms (11%). 73% of the subjects were HIV-positive, 53% had CD4 cells of 200 or below.

21% of enrolled patients were found to have culture-confirmed TB by the reference standard (solid or liquid culture). MODS was generally in agreement with the reference standard’s result 94% of the time, with a sensitivity of 85% and specificity of 97%. Positive predictive value was 88% and negative predictive value 96%. Sensitivity was higher among smear-positive subjects however, at 95%, compared to 72% among people with smear-negative disease.

The test was highly sensitive for resistance— able to detect 100% of resistant cases, with specificity of 92% of diagnosis of MDR TB. The time to results with MODS was also significantly faster with a positive culture result within a median of 9 days on MODS, compared to 16 days using MGIT liquid culture and 29 days on solid agar. The difference in turnaround time for MDR TB diagnosis was even more striking — within a median of 9 days again for MODS compared to 70 days using the reference standard.

“Although sensitivity decreased in smear-negative TB suspects, we looked at the negative predictive value in this group to determine the utility of this test for excluding active TB disease — and the negative predictive value was 97%,” said Dr Bamber. “So although sensitivity was lower among smear-negative TB suspects, MODS detected 72% of cases that would have been “missed” by smear and has a high negative predictive value in this group for confidently excluding active TB and MDR TB disease.”

Indeed, with the exception of the GeneXpert system, MODS is the only method that seems to offer much hope of rapidly diagnosing people with smear negative drug resistant TB — and in the case of HIV, that is a matter of life or death.

Scaling up laboratory capacity

According to a recent WHO guidance, MODS or other non-commercial assays might at least represent temporary options for programmes until they can introduce validated commercial assays.

[“WHO has recently endorsed the use of selected non-commercial culture and DST methods \(including MODS\), as an interim measure \(especially in resource-constrained settings\) while molecular DST capacity is being built, and under well-defined operational conditions. None of these methods are, however, suitable for decentralised use given technical complexity and biosafety concerns,”](#) Dr Weyer told HATIP.

Van Deun et al suggest that programmes may have to develop technical proficiency in a number of methods in order to provide the best coverage of a population. “Excellent coverage will only be feasible through decentralisation of simple, low-requirement methods or alternatively by centralised genotypic DST with, in principle, easy specimen referral. The small differences in DST

turnover time are relatively unimportant, provided primary culture isolation is not required,” they wrote.

Indeed, once turnaround time is shortened down from three to four months to one to two weeks, the time waiting for DST results may represent a much smaller part of the delay getting onto treatment.

In her talk on second line DST back in 2006, Dr Weyer stressed that much more is involved in setting up reliable DST services than simply mastering the technology. Clinically useful laboratory services require a transportation system to get specimens rapidly from where the suspect presents to a lab capable of performing DST, a proper plan for maintenance and supply management, staff specifically trained on culture and DST techniques, and both internal quality control and external assurance programmes in place.⁴¹ Finally, there has to be a system to get the results back from the lab as soon as they are available (and the process is pointless unless treatment is readily available).

A pilot project to scale-up DST capacity in Peru (which has chosen to implement both MODS and the NRA) has recently demonstrated Dr Weyer’s point.⁴² The project assessed the process of rolling MODS out in one region of southern Peru, while at the same time trying to identify health system and logistic challenges that could affect the utility of the new technology. The region involved 4 hospitals, 51 health centers, 182 health posts, and 38 laboratories, and some of the challenges faced were understandably related to trying to put the new system in place, in parallel with the existing system.

What was more disturbing was how patchy compliance was to existing and well-defined national guidelines for DST. “Although one-fifth of TB suspects fulfilled criteria for DST testing, cultures were sent in only 10.8% of those warranting such testing,” the researchers wrote. “In order to harness the considerable microbiological advantages of rapid, direct DST, investment must be made to ensure matching improvements in the flow of samples and results and the application of national guidelines in local practice. In addition, streamlining the DST system would likely reduce record-keeping inconsistencies (detected here for one-third of all DST requests) arising from multiple steps of information transfer and would require reallocating sample transport mechanisms.”

Indeed, it makes little sense to put new laboratory infrastructure in place, especially expensive infrastructure, without making certain that the rest of the screening, transport and treatment system for people with drug-resistant TB is in proper working order. And even in settings without functioning computer systems linking the laboratory to the clinic, given the ubiquity of cell phones/SMS technology, there really is no excuse not to develop a system for the rapid return of results.

Who to screen for TB drug resistance

Well-resourced countries and countries with a heavy burden of DR TB (more on this below) may want to routinely screen for drug resistance in everyone with TB, but countries with limited DST capacity and resources have to prioritise case detection efforts and first investigate those at greatest risk of drug resistance.⁴³

This section primarily deals with people who present with TB or are already in care — though one can make the case that the best outcomes are likely to be achieved by finding cases earlier through more aggressive case finding strategies and contact tracing.

But there may be many drug resistant cases in care, right under the clinician’s nose, and managing infection control risks in health facilities should be a consideration in where to look first. [As Dr](#)

[Krista Dong of Edendale Hospital and others have previously reported](#), there are many undiagnosed cases of TB in hospital wards — and unrecognised M/XDR TB cases in the wards could transmit the infection to other patients and staff, as was the case in Tugela Ferry.

Another aspect of case detection is that categorisation of the risk of having drug resistant TB (and whether DST capacity is rapid versus conventional) is an important determinant in whether an individual should be put on an empiric second line regimen while waiting for DST results.⁴⁴ Indeed, avoiding the expense and hassle of having someone on unnecessary second line TB treatment is one of the best reasons to develop laboratory capacity for rapid DST.

“The current WHO recommendation is that patients considered to be at risk (based on the groups listed in the Guidelines For The Programmatic Management Of Drug-Resistant Tuberculosis) be targeted first for universal access [to DST],” WHO’s Dr Karin Weyer told HATIP. “But identifying these risk groups is a country/regional responsibility, given the huge geographical variation in TB, MDR-TB and HIV epidemiology, which guides the laboratory services required.”

The DR TB Guidelines are currently being revised, however the following risk groups were highlighted in the 2008 edition, and amended somewhat in 2010’s Treatment of Tuberculosis Guidelines.

Previously treated cases:

Whether an in or out patient, once a new TB case has been diagnosed, it is extremely important to identify those who have been previously treated — especially those who have failed treatment — because they have a much higher risk of MDR-TB. (Likewise those who have failed an MDR-TB treatment regimen are more likely to have XDR-TB). The 2010 TB treatment guidelines *strongly* recommend that specimens should be obtained for culture and DST *from all previously treated patients*, defined as those who have received 1 month or more of anti-TB drugs in the past, and then further classified depending upon their outcome on their most recent course of treatment — **treatment failure, default or relapse**.⁴⁵

The prevalence of MDR-TB varies across these treatment outcomes according to a study presented at the 40th World Union Conference on Lung Health last year.⁴⁶

In this study, researchers analysed drug resistance survey data from 12 settings in 10 countries and found MDR-TB in 49% of 206 cases of treatment failure, in 32% of 208 treatment defaulters, and in 32% among 283 cases of treatment relapse.

As DST isn’t widely available everywhere yet, (even though it is one of the goals of the Global Plan to Stop TB that all previously treated patients have access to DST by 2015), classifying previously treated individuals into these patient registration groups can be helpful in settings with limited DST capacity, with the caveat that local drug resistance surveys should also be conducted to guide screening decisions since the prevalence of resistance within previously treated individuals varies by setting.

It is worth noting that the highest rate of MDR-TB — exceeding 80% in some settings — has been reported among those who have failed what was previously referred to as the category II regimen (a slightly beefed up and lengthier version of the first-line TB regimen), that was widely recommended for people failing or relapsing after their first course of TB treatment.⁴⁷ Although WHO’s guidelines suggest this retreatment regimen may still be used for people who have relapsed or defaulted first-line treatment in settings without DST capacity (provided that local surveys suggest drug resistance is

not very common in these subcategories), the regimen is unnecessary for cases with drug susceptible TB and ineffective against cases of MDR-TB.

In fact, using this regimen in people with MDR-TB may just lead to the evolution and onward transmission of more resistant strains of TB. Consequently, WHO intends to phase out the regimen once DST becomes widely available.⁴⁸

Other aspects of the individual’s treatment history (such as a known pattern of poor treatment adherence, or a history of relapsing or defaulting more than once) as well as *where* he or she was treated could indicate that a person is more likely to have drug resistance. For instance, receiving TB treatment in the private sector in many settings, or from a poorly operated TB programme (where for instance, there have been frequent drug stock-outs), or where the anti-TB drugs have been found to be of poorer quality have all been associated with the development of MDR-TB.⁴⁹

People with active TB failing their current regimens

also have a higher risk of drug resistant TB — though failure could also be due to simply not taking their treatment in settings with inadequate treatment support. In new or retreatment cases, WHO recommends that if a specimen obtained at the end of month three is smear-positive (which, in new patients, is generally only collected if the specimen at the end of month two was positive), sputum culture and drug susceptibility testing (DST) should be performed.

The guidelines do not recommend sending out for DST if the sputum is smear-positive at the end of month two because “available evidence showed that smear status at the end of the intensive phase is a poor predictor of relapse, failure and pretreatment isoniazid resistance.” (One reason for this is that people can continue to produce non-viable bacilli which will show up on microscopy, but which cannot be cultured.)

However, local policy varies — for instance, in the Western Cape of South Africa, the policy is to send out for DST when cases are still smear-positive at the end of month two. Again, the time it takes to get DST results back and the risk of M/XDR-TB transmission in the community are important considerations since the person may be failing the first line regimen because they had M/XDR-TB to start with.

Contacts of people known to have M/XDR-TB:

After treatment failures, the risk of resistance is probably greatest among people who develop active TB after contact with a person with documented M/XDR-TB — especially close household contacts. WHO recommends that anyone who develops TB after contact with someone with M/XDR- should be treated with a regimen based on the DST of the presumed source case while awaiting DST results.

However, transmission of drug resistance cannot be taken for granted when there is a high burden of TB in the community. Again, in such cases, it is important to have rapid DST results because second line regimens are dramatically more expensive, more toxic, and less effective for people with drug susceptible TB.

Similarly, people who develop TB after exposure in institutions with MDR-TB outbreaks need DST results as soon as possible; and depending on the setting, there may also be an increased risk of drug resistance after hospitalization or incarceration (DST should also be prioritised for staff members who develop TB after working in those facilities).

This means, of course, that people diagnosed with TB need to be asked carefully about whether they have had any known contact with people with TB, or possible exposure to TB in congregate settings.

Everyone with HIV

: Not only do people with HIV have an elevated risk of exposure to M/XDR-TB (because of their increased utilisation of the health system, and possibly other risk factors) they are in greatest need of rapid M/XDR-TB diagnosis because M/XDR-TB can be rapidly fatal.

“WHO recommends that NTPs undertake DST at the start of TB therapy in all HIV-positive TB patients, to avoid mortality due to unrecognised drug-resistant TB, and strongly encourages the use of rapid DST in sputum smear-positive persons living with HIV,” according to the 2010 Guidelines.

In settings where the capacity does not yet exist to provide DST for everyone with HIV-related TB, the guidelines suggest first screening people who have failed treatment, then those who relapse or have defaulted on treatment. In addition, it may also be wise to prioritise people with lower CD4 cell counts.

A word about smear negative M/XDR-TB in people with HIV

. Given the poor sensitivity of molecular tests in smear-negative TB, WHO recommends that conventional DST be performed in these cases, or a rapid DST should be performed on a cultured isolate as soon as it is available.

However, smear microscopy misses huge numbers of people with HIV and TB — and one of the most effective ways to diagnose these cases, the smear-negative TB diagnostic algorithm, depends on a response to TB treatment — a response that won't be seen in people with HIV and drug resistant smear-negative TB (although there is a chance of eventual diagnosis if a specimen was sent in for culture).

But in reality, this means that diagnosis won't come in time in the majority of those who have the greatest risk of mortality from M/XDR-TB. This is unconscionable. It is unreasonable to not find an alternative DST solution for people with HIV and smear-negative M/XDR-TB — expecting them to wait for conventional DST simply is not acceptable. For this reason, either the GeneXpert system or MODS simply *must* be scaled up in settings with a high burden of HIV and DR TB, until a better (cheaper, safer, more sensitive) rapid test is found.

People with other co-morbid conditions:

A number of other illnesses have also been associated with M/XDR-TB or a higher risk of mortality from it, such as malabsorption and diarrhoea and Type 2 diabetes.⁵⁰

All new TB cases, in settings with a high rate of M/XDR-TB transmission

As already noted, in settings with a high burden of M/XDR-TB in new TB cases, the risk of ongoing transmission, morbidity and mortality is simply too great to not screen everyone. But what exactly this threshold should be is subject to debate. The 2008 DR TB Guidelines, and the 2010 TB Treatment Guidelines suggest that if country data (or WHO estimates) show that more than 3% of new patients have MDR, DST should be obtained at the start of therapy for all new patients.

Countries such as South Africa don't quite meet these criteria and so new cases are only screened after they appear to be failing treatment.

But many clinicians and researchers speaking at the 2nd South African TB conference made strong cases for screening all new TB cases in that country. One was Dr Cheryl McDermid, from MSF in Khayelitsha, who described the findings of a DR-TB survey in 2008 to illustrate what the implications of the policy would be. The survey

found that 5.2% rifampicin resistance was found among new cases and 11.1% in previously treated cases (rifampicin resistance was being used as a rough surrogate for MDR-TB).

“When people look at this data, they think we need to focus on previously treated TB cases because rifampicin resistance is higher in previously treated cases. However when we look at what is actually seen in Khayelitsha we find many more new cases notified - 4279 new cases notified compared to 1512 retreatment cases. And in absolute numbers, the majority of rifampicin resistance is amongst new cases.”

She estimated that about 54% of the cases were detected that year in Khayelitsha — and said that the number of cases (not simply the cases being detected) keep going up despite a good TB control programme in Khayelitsha. 61% of the cases who died of drug-resistant TB in the cohort were “new” cases and all were HIV-positive.

“Despite dramatically improved case detection in Khayelitsha, case detection is still not high enough to interrupt transmission,” she said. “So improved case detection and reduced mortality AND reduced transmission culture and DST for all TB suspects is an urgent priority.”

In the next iteration of the Guidelines for DR TB, there may be a shift away from the 3% threshold.

“One of the questions in the forthcoming guidelines will be thresholds of DR prevalence for the promotion of wider testing with rapid techniques like LPA. Apart from South Africa other countries are scaling up in the use of these diagnostics, such as Lesotho and Ethiopia,” Dr Dennis Falzon of WHO told HATIP.

“My personal feeling is that there is no ‘magical’ threshold for rapid resistance testing. The best we can do is to model the cost-effectiveness and cost-benefit of various approaches, and policy decisions would then have to be based on available resources,” said Dr Weyer.

Active case finding and contact tracing for M/XDR-TB

While health workers should, of course, first focus on providing care for the clients they have on hand, given the extremely poor outcomes in southern Africa in people who present for care with M/XDR-TB, programmes may have a better chance of reducing mortality (and transmission) by identifying and providing care for those cases that have yet to be identified.

For example, [a household contact tracing study conducted in the Msinga sub-district, where the Church of Scotland Hospital in Tugela Ferry is located](#), reported dramatically better survival among the household contacts it identified with M/XDR-TB than among the index cases, with mortality of only 14% and 52% among MDR-TB and XDR-TB household cases, respectively, within a median of 506 days of follow-up.⁵¹

In other words, presuming recommended treatment and care are available, the sooner a case of M/XDR-TB is identified and diagnosed, the better will be the outcome — perhaps profoundly so. Since people with HIV and drug resistant TB have so little time to wait, it is particularly important to actively look for cases at high risk of having M/XDR-TB and among people with HIV.

Resources

Unitaid

<http://www.unitaid.eu/en/>

FIND (diagnostics)

<http://www.finddiagnostics.org>

MSF's Report on TB Diagnostics and DST

http://www.msfaccess.org/fileadmin/user_upload/diseases/tuberculosis/Diagnostics%20Pipeline%20Report.pdf

WHO Guidelines for surveillance of drug resistance in tuberculosis

http://whqlibdoc.who.int/publications/2009/9789241598675_eng.pdf

WHO treatment of TB guidelines

http://www.who.int/entity/tb/publications/tb_treatmentguidelines/en/index.html

WHO Guidelines for the programmatic management of drug-resistant tuberculosis: Emergency update 2008

http://www.who.int/entity/tb/publications/2008/programmatic_guidelines_for_mdrtb/en/index.html

The Project Greenlight Committee

<http://www.who.int/tb/challenges/mdr/greenlightcommittee/en/index.html>

WHO Global Laboratory Initiative (GLI)

<http://www.who.int/tb/dots/laboratory/gli/en/index.html>
 WHO policy statement: molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis
 WHO policy statement: Non-commercial culture and drug-susceptibility testing methods for screening of patients at risk of multidrug-resistant tuberculosis [pdf 328kb]

Stop TB Partnership Working Group on New Diagnostics

http://www.stoptb.org/wg/new_diagnostics/

Pathways to Better Diagnostics of TB

http://www.stoptb.org/wg/new_diagnostics/assets/documents/BlueprintTB_annex_web.pdf

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