

Future prospects for the MODS assay in multidrug-resistant tuberculosis diagnosis



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'...a potentially useful tool for harnessing the impressive outcomes achieved by the DOTS-plus strategy for the management of MDR TB.'

Extensively drug-resistant tuberculosis In 2004/2005, workers in Kwazulu-Natal, South Africa, became aware that some patients receiving effective antiretroviral therapy in their pioneering integrated HIV/tuberculosis (TB) program were dying despite undetectable HIV viral loads. Their subsequent detailed epidemiological study with enhanced surveillance for TB drug resistance finally blew the lid off an issue that has been smouldering unspoken for too long – that the era in which standardized, 'best guess', empirical TB therapy was an adequate blanket approach to TB control is over. Gerry Friedland and colleagues uncovered a previously unnoticed outbreak of extensively drug-resistant (XDR) TB [1], the current definition of which is the occurrence of TB in persons whose *Mycobacterium tuberculosis* isolates are resistant to isoniazid and rifampicin (thus all XDR is also multidrug-resistant [MDR]) and also resistant to any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin or capreomycin) [2].

'...we simply cannot predict with confidence which patients will have MDR disease.'

The emergence of data such as that from Kwazulu-Natal should come as little surprise – global surveillance had already demonstrated that XDR TB was present in all WHO regions and that 4% of MDR strains from the USA were XDR [3]. Indeed, in the USA, this data was readily available because, in common with Western Europe and other industrialized nations, drug susceptibility testing (DST) is the standard of care; a patient with microbiologically confirmed TB always has DST performed as a routine. And yet in a classic example of the 'inverse care law' [4],

this standard of care has hitherto been regarded as unsuitable for the highest burden countries where resources are almost invariably scarce.

First do no harm

Not only is this iniquitous double-standard offensive but ignoring the microbiology has now come back to haunt us. One of the pillars of the directly observed treatment short-course (DOTS) strategy is direct observation of patients taking their medicines; in most settings this requires that patients attend the health center every day, often spending at least 20–30 min in the company of other patients and healthcare staff. In many countries a significant proportion of those patients and some of the staff will also be HIV co-infected. A patient with undetected MDR TB receiving ineffective therapy will continue to attend the health facility every day for treatment until, after 3 or 4 months of deteriorating health, the illness threshold is surpassed and a DST is requested. After a delay of at least 2 more months (and often more like 6 months) the DST result will come back indicating MDR disease. During this prolonged period all those patients that were being cured of their original drug-susceptible disease and the healthcare staff attending them, will have been highly exposed – those that do not develop early MDR disease will have laid down a time bomb of latent MDR infection for the future. This is probably what occurred in Kwazulu-Natal with widespread institutionally centered transmission of a highly resistant strain. Because so many of the affected patients were HIV co-infected and quickly developed the disease rather than the latent infection phenotype, the extensive transmission was effectively unmasked. The outbreak also drove home the hard lesson that we simply cannot predict with confidence which patients will have MDR disease. A high odds ratio will help us know that a patient who has previously failed therapy is at high risk but the majority of MDR patients have no such identifiable risk factor to alert clinicians to the need for DST. In Kwazulu-Natal, very few of the XDR patients had recognized risk factors other than the fact that they were attending a health facility.

This outbreak is surely only the tip of a very large iceberg of nosocomial transmission associated with clinic-based direct observation. The implications of this situation for the concerted drive towards integration of HIV and TB services are considerable and warrant further thought and debate. Although the collaborative approach has distinct advantages (cross-training of staff leads to higher index of suspicion and thus likelihood of testing for TB in HIV patients and vice versa, availability of antiretroviral therapy for TB/HIV co-infected patients) deliberately bringing together the patient group most susceptible to TB disease (HIV patients) with TB patients whom we hope rather than know we are rendering noninfectious through appropriate therapy might not necessarily be the optimal strategy if infection control measures are not strictly enforced.

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DOTS-plus & the case of the missing microbiology

Considerable success has been demonstrated by the DOTS-plus strategy for management of MDR TB [5], particularly when a community-based approach is adopted [6]. The heavily discounted second-line drug costs and procurement channels facilitated by the Green Light Committee enable an efficient and effective system for addressing this difficult problem in settings where resources would usually not permit action. But the DOTS are not quite joined up – urgent scale-up is limited by MDR diagnostic capacity. In the industrialized world DST means indirect susceptibility testing of known inocula of cultured strains of *M. tuberculosis* against all first-line therapeutic agents, usually rifampicin, isoniazid, ethambutol, streptomycin and pyrazinamide. In programmatic conditions the key susceptibility information of interest is whether the patient harbors MDR or non-MDR disease – a change in therapy in response to any other resistance profile is unlikely. Thus, testing for ethambutol (notoriously tricky [7]), streptomycin and pyrazinamide (complicated by pH requirements [8]) is redundant in the initial screen and best saved until DST for second-line drugs is performed for identified MDR strains.

MODS: an entry point for MDR & XDR DST
The microscopic-observation drug-susceptibility (MODS) assay was developed at Universidad Peruana Cayetano Heredia in Lima by Luz Caviedes and Bob Gilman whilst working on colorimetric redox indicators. When Luz noted that by examining cultures in 24-well tissue culture plates with an inverted light microscope she could clearly identify colonies of *M. tuberculosis* in broth long before the growth effected a color change, Bob immediately recognized the potential utility of this finding for development of a diagnostic assay. That *M. tuberculosis* grew in this characteristic cording pattern had been known for decades but the proposed incorporation of TB drugs from the outset to perform direct DST was generally regarded with scepticism [9]. Traditionalists understandably preferred to be able to control the inoculum by preparing suspensions of known mycobacterial concentration (MacFarland 1) and doubted that the range of inoculum concentrations present in concentrated, decontaminated sputum samples would permit robust DST. It turns out that for ethambutol and streptomycin they may well have been right [10], and further work is needed if the MODS assay is to prove useful for testing against these agents. However, when field tested against a rigorous double gold-standard of automated MBacT DST and proportion method DST (with discrepant analysis using minimum inhibitory concentrations [MICs] from the microplate-based alamar blue assay [MABA] [11]) the direct MODS assay generated rifampicin and isoniazid susceptibility results that were highly accurate [12]. Moreover, these MDR results were available in a median of 7 days, compared with 22 and 68 days, respectively, for the two reference methods. This method demonstrates rapid, accurate detection of MDR TB and all at a cost of under US\$2 per sample (excluding labor). As all XDR TB is by definition MDR, the MODS assay is a potentially useful tool for screening populations to detect XDR – MDR isolates that are rapidly available from drug-free control wells can be used for second-line DST.

Streamlining the MODS methodology

In the same study a certain amount of redundancy in the original MODS assay was noted and the new, streamlined assay is now simply a four-well per sample affair – two drug-free control wells and one well each containing rifampicin and isoniazid. The logic of this reduction from the previous 12-well format is that [13]:

- Inclusion of two different concentrations for each drug added no additional information than one concentration alone for rifampicin and isoniazid
- Wells for ethambutol and streptomycin have been dropped
- Growth in two drug-free control wells provides sufficiently high specificity such that cross-contamination is a very unlikely cause

Bacterial & fungal overgrowth

The increased sensitivity of liquid culture is also its Achilles heel – bacterial and fungal overgrowth are more frequent than with less sensitive solid media culture. Approximately 8% of MODS cultures are affected by fungal or bacterial overgrowth, which usually occurs early and is notable by the time of the first plate reading on day 5. After decontaminating the stored aliquot of ‘backup’ sputum sample a second time the culture can be quickly set up again and only in less than 0.5% of all samples can a final result not be established. The way in which the assay is designed permits definitive results to be reported, sometimes even if bacterial overgrowth occurs. For example, if control wells are negative then overgrowth in drug-containing wells is of no consequence and if mycobacterial growth can be distinguished in both drug-free wells even in the presence of fungal or bacterial overgrowth then a culture can be regarded as positive and the drug-containing wells can be read if they are free of contamination. Clearly on occasion a MODS culture is positive but the DST cannot be read – fortunately this occurs rarely.

‘...only in less than 0.5% of all samples can a final result not be established.’

Biosafety

Proposing that developing world laboratories should manage liquid TB culture generates a spinal reflex of anxiety in traditional mycobacteriologists. Not without good reason. Liquids spill, solids don’t. Liquids aerosolize, solids tend not to. However, conventional liquid culture systems require either puncture of bottle caps to assess growth or subculture of liquid media teeming with mycobacteria at concentrations many logs greater than the initial sputum sample. Manipulating these liquids to obtain the correct concentration for inoculation is fraught with risk of aerosolization and spillage and thus both culture

cross-contamination and occupational infection. This is where the direct DST element of MODS plays its second trump card. The MODS plate, once inoculated with the clinical sample and culture media, is sealed inside a transparent ziplock plastic bag. It is not opened again and no manipulation of the culture material takes place so the magnification of infectious material that takes place in the liquid media remains sealed safely away until the whole is autoclaved prior to disposal. Safer than any culture method, liquid or solid, that requires manipulation of cultured strains and indirect DST.

Doing it

The perennial question that I am asked when describing MODS is ‘So why are we all not doing it?’ Why indeed. The MODS methodology is effectively ‘laboratory freeware’ in that there is no punitive intellectual property position and there are no hidden secrets – all consumables are freely available from well-known laboratory suppliers and there are no magic ingredients in Middlebrook 7H9, OADC and PANTA (see MODS user guide at [101]). The safety issue should now be put to bed. Prolonged training requirement is often cited as an obstacle, although our growing experience in training visitors suggests this is unwarranted. Training qualified laboratory personnel to set up the MODS assay takes 2 days on site, but in reality simply following the standard operating procedures in the user guide should suffice, and on-site training, although desirable, is probably not essential. Learning the pattern recognition element of plate reading takes between 2 and 5 days and should be supplemented by reference to a library of photo images – the pattern of growth evolves from day to day and varies depending upon the sample bacillary load so that it is useful for trainees to read over a hundred plates to gain an appreciation of the range and variety of appearances as well as to be familiar with the appearance of nontuberculous mycobacteria and fungal and bacterial overgrowth. Newly trained novice technicians can read a well in one minute, much quicker than a sputum acid-fast bacillus smear or malaria film. If a novice is uncertain whether what they are looking at is the earliest sign of growth (tiny commas) or just sample debris then they can simply replace the plate in the incubator and have another look the next day to see if the characteristic cording structures are starting to develop. Although to date our approach has been to recommend on-site training, the recently published experience of

three separate groups who taught themselves MODS using standard operating procedures and published articles indicate that some laboratories can simply pick up the methodology and run with it [14–16].

Conclusion

There has long been a moral need to improve TB diagnostic capacity in high-burden resource-limited settings. Recent recognition of the growing importance of MDR and now XDR disease has upped the stakes considerably and the imperative is generally now widely accepted. Amongst the new diagnostic tests that could help to address these emerging twin epidemics is the MODS assay. Developed specifically with the developing world in mind, and recently streamlined after thorough field evaluation to retain all the strengths and shed all redundancy from the original methodology, the MODS assay now comprises a relatively simple four-well test. High sensitivity and specificity, marked speed and concurrent, accurate, rapid MDR testing all for less than US\$2 mark MODS out as a potentially useful tool for harnessing the impressive outcomes achieved by the DOTS-plus strategy for the management of MDR TB.

Future perspective

The MODS methodology is ready to use, much as Löwenstein-Jensen culture currently exists, but for effective scale-up and implementation to a more peripheral health facility level the evolution of a simple off-the-shelf kit form that may not require an inverted light microscope is a realistic goal. Furthermore, having a test in the laboratory is only one piece of the jigsaw. Determining the optimal way in which the test should be used in order to maximize the epidemiological footprint left by implementation (as measured by reduction in disease incidence) in the most cost-effective and affordable manner are current knowledge gaps that must be addressed if policy-makers are to be persuaded to use MODS. Although this paper has focused on MODS as an MDR TB detection tool, it should not be forgotten that, in common with most culture-based diagnostics, MODS effectively doubles case ascertainment achieved by sputum smear microscopy, the most widely used method in the world today, even when only one specimen is sampled [12]. Bringing culture to settings where it has previously been unavailable for economic, technical or systematic reasons could considerably alter the epidemiological landscape – only operations research will enable us to quantify the impact.

Executive summary

- The microscopic-observation drug-susceptibility (MODS) assay was developed for rapid, accurate detection of tuberculosis (TB) in sputum with equally quick direct drug susceptibility testing.
- The MODS assay provides low-cost, safe and sensitive detection of TB faster than existing gold standards and automated methods with concurrent highly accurate identification of multidrug-resistant (MDR) strains.
- Training requirements are modest and there is realistic potential for future simplification for wider scale-up.
- Optimally cost-effective implementation strategies need to be defined, as for all new diagnostic tests.
- The MODS assay brings the opportunity for high-performance TB and MDR TB diagnostic testing to the most afflicted settings, which have hitherto been least capable of accessing such resources. The inverse care law may yet be reversible.

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Website

101. MODS user guide
www.upch.edu.pe/facien/dbmbqf/mods/mods.htm

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