

## INTRODUCING MODS: A LOW-COST, LOW-TECH TOOL FOR HIGH-PERFORMANCE DETECTION OF TUBERCULOSIS AND MULTIDRUG RESISTANT TUBERCULOSIS

L Caviedes, \*DAJ Moore

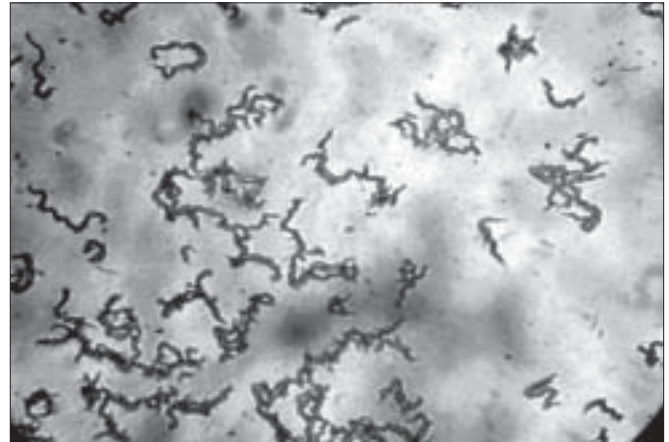
The urgent need for improved diagnostic tools for detection of tuberculosis (TB) and multidrug resistant tuberculosis (MDRTB) has been repeatedly highlighted. It is patently absurd that in 2006 the vast majority of global tuberculosis is diagnosed by means of a century-old method, which though inexpensive and simple, fails to detect half of all cases tested. That there are sharper tools which exist yet are beyond the reach of the healthcare systems and patients most in need of them, is an insult to us all and we should be ashamed of this global inequity in TB diagnostics.

To have an impact upon TB control and patient care in resource-limited high TB burden settings any new tool will need to be affordable, rapid, robust and technically straightforward. One potential candidate might be MODS<sup>1-3</sup> - the microscopic observation drug susceptibility assay - which has been developed and evaluated in Peru specifically with these requirements in mind.

### MODS

MODS depends upon three key principles (which have been known for decades): (1) *Mycobacterium tuberculosis* grows faster in liquid (broth) than on solid media, (2) in liquid cultures *M. tuberculosis* grows in a visually characteristic manner (tangles, cording) which can be observed under the microscope long before the naked eye could visualize colonies on solid agar (Figure), (3) incorporation of anti-TB drugs into broth cultures at the outset enables direct susceptibility testing from sputum samples.

Thus, MODS is a tissue-culture plate based assay utilising observation of Middlebrook 7H9 cultures with an inverted light microscope to detect the characteristic tangles of *M. tuberculosis* in liquid media. Drug-containing and drug-free control wells allow concurrent drug susceptibility testing (DST) for rifampicin and isoniazid. The Middlebrook 7H9 medium is supplemented with antimicrobial and nutritional supplements (PANTA and OADC respectively) and sputum



**Figure:** Characteristic tangles of *Mycobacterium tuberculosis* in MODS culture plate (20x objective)

samples are previously decontaminated by the NALC-NaOH method. All consumables and reagents are available from standard laboratory suppliers - MODS is a methodology usable by all, not a product. The plates are contained within ziplock polythene bags for safety and are read under the microscope daily (or on alternate days if preferred) from day five. If growth is observed in the drug-free control wells then the isoniazid and rifampicin containing wells are read simultaneously. Concurrent growth in drug-containing wells indicates resistance.

### Evolution

MODS arose during experiments conducted by Luz Caviedes under the guidance of Professor Robert Gilman at Universidad Peruana Cayetano Heredia in Lima, Peru in the late 1990s in which a colorimetric test for TB growth was being investigated. The observation that microcolonies could be seen under the microscope long before a colour change occurred prompted the development of MODS. Recently completed operational field studies have served to refine and streamline the methodology further and importantly validate MODS as a test for TB detection and MDRTB detection directly from sputum.

### Data

The key data are as follows: the sensitivity of detection (98%) significantly exceeds automated MBBacT (BacTAlert) culture (89%) and Lowenstein-Jensen (84%) and renders

\*Corresponding author (email: <davidajmoore@msn.com>)  
Imperial College London Wellcome Trust Centre for Clinical Tropical Medicine (LC) and Laboratorio de Investigación de Enfermedades Infecciosas (DAJM), Universidad Peruana Cayetano Heredia, Av. Honorio Delgado No 430, Urb. Ingeniería, Perú  
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a second MODS culture of very limited benefit; speed of detection (median seven days) significantly exceeds MBBacT (13 days) and LJ (26 days) - add a further nine and 42 days respectively for susceptibility testing by MBBacT and proportion method, but none for MODS.<sup>1</sup> All positive cultures are detected within 21 days and >98% within two weeks thus a negative MODS culture at three weeks is a confident rule-out. MODS delivers 99% concordance for MDR testing with gold-standard comparators. Material and running costs (excluding labour) for detection and MDR testing are currently US \$2 per sample, approximately one twentieth the cost of MBBacT.

#### Training and workload

The methodology is straightforward and the SOP simple to follow. Two weeks training is more than sufficient, about half of which is spent learning to differentiate very early *M. tuberculosis* growth from debris - in reality this simply allows recognition one or two days prior to the more characteristic unmistakable tangling appearance which is quickly recognised and learned. We know of one laboratory in Ethiopia, which started to use MODS simply by following the SOP without any face-to-face training and managed to achieve performance very similar to that described above (median time was nine days) with very low bacterial/fungal contamination rates (<1%) (personal communication, Girum Shiferaw), (SOP available at [www.imperial.ac.uk/medicine/people/daj.moore](http://www.imperial.ac.uk/medicine/people/daj.moore)). This and other anecdotal reports from Peru indicate that users find MODS considerably simpler to perform and associated with a significantly lesser workload than conventional cultures with subsequent indirect DST (personal communication Cesar Augusto Rojas). Culture contamination occurs at about 50% the rate of Lowenstein Jensen slopes inoculated concurrently; moreover contamination is swiftly apparent as the media clouds over within five days so the remaining sample can be re-decontaminated and re-plated within one week - further contamination thereafter is extremely rare.

#### Biosafety and equipment

Legitimate concerns about biosafety with other liquid culture systems do not really apply to MODS, indeed the converse is the case. After inoculation with decontaminated sample the MODS plates are permanently sealed in ziplock polythene bags through which the microscopic examination is made, thus spillage of the mycobacterial “soup” cannot occur. Furthermore, as no secondary sub-culture is needed (because this is direct and not indirect susceptibility testing) no further manipulation is required - this zero potential for aerosolisation or accident compares favourably with the hazard associated with preparation of a standardized inoculum for indirect DST. An indirect indication of how rarely dispersion of infectious material occurs is the very low frequency of cross-contamination.<sup>4</sup> However, as for all mycobacterial culture work, a biological safety cabinet is recommended. The other required equipment for MODS, which would usually be available in an existing TB culture

laboratory, is a fridge/freezer for storing media, a vortex and centrifuge for sputum decontamination, an incubator and an autoclave. An inverted light microscope is the only required item which many laboratories lack and thus requires an initial capital outlay (around US \$3500 minimum).

#### MDR testing vs. full first-line DST panel

Although it is a common practice, when doing DST, to test against all first line drugs, the only susceptibility data likely to alter management in a TB programme is identification of MDR disease and thus rifampicin and isoniazid alone should suffice. If MDR is identified then ethambutol, pyrazinamide and streptomycin testing can accompany subsequent second line DST - this information is otherwise (i.e., in the absence of MDR) relatively redundant. The drug-free control wells in MODS provide a ready-cultured strain for this testing by a second line panel, further speeding up the DST process.

#### Learning the lessons of roll-out

The establishment of a new test in any laboratory requires more than just turning up with the gear and the recipe. The need for training, evaluation, quality control and the demands on staff time, laboratory space and institutional systems for handling samples, issuing results and ordering materials should be considered. As MODS roll-out starts to take shape we are learning all the time how to better effect this translation and optimise strategies of implementation, lessons which should be useful to all endeavouring to bring high quality new TB diagnostics to the field, to finally start to address the iniquitous inverse care law wherein the highest quality diagnostic tools are least available to the settings with the greatest need.<sup>5</sup>

#### References

1. Moore DA, Evans CA, Gilman RH, Caviades L, Coronel J, Vivar A, *et al.* Microscopic-observation drug-susceptibility assay for the diagnosis of TB. *N Engl J Med* 2006;**355**:1539-50.
2. Moore DA, Mendoza D, Gilman RH, Evans CA, Hollm Delgado MG, Guerra J, *et al.* Microscopic observation drug susceptibility assay, a rapid, reliable diagnostic test for multidrug-resistant tuberculosis suitable for use in resource-poor settings. *J Clin Microbiol* 2004;**42**:4432-7.
3. Caviades L, Lee TS, Gilman RH, Sheen P, Spellman E, Lee EH, *et al.* Rapid, efficient detection and drug susceptibility testing of *Mycobacterium tuberculosis* in sputum by microscopic observation of broth cultures. The Tuberculosis Working Group in Peru. *J Clin Microbiol* 2000;**38**:1203-8.
4. Moore DA, Caviades L, Gilman RH, Coronel J, Arenas F, LaChira D, *et al.* Infrequent MODS TB culture cross-contamination in a high-burden resource-poor setting. *Diagn Microbiol Infect Dis* 2006;**56**:35-43.
5. Hart JT. The inverse care law. *Lancet* 1971;**1**:405-12.