

Biosafety and MODS

A misconception has arisen about the perceived laboratory biohazard associated with the MODS methodology for TB and MDRTB diagnosis¹. This is partly because MODS utilises liquid media and partly because the full procedure has not been clearly understood.

Contrary to this misconception we firmly believe that MODS is actually considerably safer than all indirect TB drug susceptibility methods.

This is why.

The hazard of positive cultures in liquid media arises from spillage risk and manipulation of cultures teeming with organisms.

Conventional wisdom recognizes that liquid TB culture media (such as that used in MODS, MGIT, BACTEC and MBBacT systems) pose a greater biohazard than solid media because of the risk of spillage, aerosolisation and (less importantly) larger mycobacterial loads.

Manipulation of positive liquid cultures is particularly hazardous². Indeed, this essential element of the process for performance of standard indirect (secondary) drug susceptibility testing (DST), involves the handling of mycobacterial loads thousands of times greater than that found in pre-inoculation clinical specimens, thus dwarfing the risks associated with the handling of sputum samples.

MODS utilises direct DST thus involves no culture manipulation

As a methodology utilising **direct** DST, MODS actually results in considerably less biohazard exposure risk than any method requiring isolate manipulation.

Spillage risk is nullified by handling MODS plates in zip-lock bags

Because inoculated culture plates are sealed within polythene ziplock bags from which they are never removed (microscopic examination is done through the transparent bags), the culture amplification of *M tuberculosis* and MDR testing effectively occurs within a closed system. The only consequence of a dropped plate is a spoiled culture as any spillage is completely contained.

Biosafety requirements - P3 is neither necessary nor appropriate

Biosafety level 3 standards require, amongst other things, a ventilation system with uni-directional airflow, external exhausting of laboratory air and careful continuous maintenance. The risks associated with ventilation systems which fail are well known and increasing complexity demands increased maintenance expertise and costs which are prohibitive in the resource-limited settings where TB is most prevalent. Safe TB culture requires:

1. a well-organised laboratory with a biological safety cabinet (BSC)
2. appropriate protective clothing and NIOSH approved respirators used at all times by laboratory workers

The risks associated with TB laboratory work relate primarily to sample preparation, culture manipulation and waste disposal. Culture

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manipulation is by far the most hazardous of these as highly concentrated aliquots of mycobacteria are exposed to the laboratory air; thus a call for P3 facilities might be justified² when indirect DST is performed.

However this does not apply to MODS for which a well-positioned and properly maintained class II BSC re-circulating exhausted HEPA filtered air into a closed room is more than adequate. This cleans room air and provides a safe environment at lower capital and maintenance cost, more than adequate for the MODS methodology in which the only potential period of risk is in sample preparation for plate inoculation.

1. Moore DA, Evans CA, Gilman RH, et al. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. *The New England journal of medicine* 2006; 355(15):1539-50.
2. Iseman MD, Heifets LB. Rapid detection of tuberculosis and drug-resistant tuberculosis. *The New England journal of medicine* 2006; 355(15):1606-8.

See also:

Moore DAJ, Gilman RH, Friedland JS. MODS assay for the diagnosis of TB. N Engl J Med 2007; 189

Frequently Asked Questions

1. How much will MODS cost me?

Material costs are \$2 per sample. This includes taking into account the need for repeat processing of contaminated cultures and is based on 5 samples being processed per 24-well plate.

Using labour costs in Peru the per sample cost for a laboratory processing 5000 samples per year is approximately \$2.30. Capital set-up costs depend upon existing infrastructure

2. What equipment is needed to do MODS and where can I get it?

A fridge/freezer is needed to store pre-prepared broth and antibiotic stocks; a vortex helps to aid the sputum decontamination process; a biosecure centrifuge (which need not be refrigerated if it is large enough to be only required for one usage per day) for sputum concentration; an incubator (which need not be CO₂ enriched) for culture; an inverted light microscope to read MODS plates – this is the item most commonly lacked by implementing laboratories; an autoclave to sterilize media, PBS and used plates; a balance to weigh isoniazid, rifampicin and NALC; a 4-channel multipipette.

3. Where can I buy a MODS kit?

You can't. Or at least not yet. Developmental work and preliminary experiments are underway in collaboration with PATH to explore the possibility of a MODS kit in the future.

4. Where can I get the laboratory materials that I need?

Because we are asked this question a lot we have included information on laboratory suppliers (catalogue numbers etc.) in the user guide. However, there may be other more suitable suppliers in your region. The quality of the reagents is obviously very important and good growth of the positive controls in the assay is essential to demonstrate that the media procured support growth of *M tuberculosis*.

5. I'm worried about the safety of liquid culture – should I be?

No, we don't think so. See the detailed discussion of this issue under **Biosafety and MODS** above. Unless strains are being manipulated we see absolutely no need for level III biosafety; level II is completely adequate.

6. What training do I need to do MODS?

Our favourite anecdote is of Girum Shiferaw who wrote to us from Addis Adaba (Ethiopia) for the MODS SOP a few years ago. The next thing we knew he was presenting the results of his work at the Annual Conference of the International Union Against TB and Lung Disease! Prior to this we had perceived that at least one week of on site training was needed (preferably two) to learn the basic preparatory steps and the pattern recognition that is integral to detection of *M tuberculosis*. Whilst we still think that this is optimal and are working towards establishing a network of MODS training laboratories in Africa and Asia, it is clear that it is not

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essential for everyone (see Girum's paper in the bibliography). We also hope that this website proves to be a valuable resource and that the photos provided can be printed out as aide-memoire posters for the laboratory wall.

7. How do I know if MODS is working in my hands? What quality control is there?

This is described in the **User Guide**. The internal quality controls are designed to assure that (1) the media mix (Middlebrook 7H9, OADC and PANTA) supports mycobacterial growth [the positive control strains inoculated into drug-free wells on a separate plate]; (2) the rifampicin and isoniazid wells contain the correct concentration of active drug to distinguish between susceptible and resistant *M tuberculosis* [the susceptible and MDR strains inoculated on the positive control plate]; (3) that cross-contamination is detected [the four sample-free negative control wells on every MODS plate].