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# Clinical evaluation of the microscopic observation drug susceptibility (MODS) assay for detection of *Mycobacterium tuberculosis* resistance to isoniazid or rifampin

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**ABSTRACT**

This prospective study evaluated performance of the microscopic observation drug susceptibility (MODS) assay for direct detection of *M. tuberculosis* drug resistance. MODS sensitivity, specificity, and positive and negative predictive values were 96.7% (95% C.I. 92.1-98.8), 78.4% (73.5-80.6), 82.4% (78.4-84.2), and 95.8% (89.9-98.5) respectively for isoniazid resistance, and 96.0% (90.3-98.6), 82.9% (78.8-84.7), 80.0% (75.2-82.1), and 96.7% (91.9-98.8) respectively for rifampin resistance. For both rifampin and isoniazid testing, the likelihood ratio of a negative test was ≤ 0.05, indicating that MODS may be useful for "ruling out" drug resistance.

Approximately 424,000 cases of multidrug-resistant (MDR) tuberculosis (TB) are estimated to have occurred in 2004 (12). Accordingly, the new *Stop TB Strategy* and *the Global Plan to Stop TB, 2006-2015* include MDR-TB management as a basic component of TB control (10,11). Recognition of a cluster of patients with HIV-associated, extensively drug-resistant TB (XDR-TB) in KwaZulu-Natal Province, South Africa further underscores the need for prompt diagnosis of drug-resistant TB, since mortality was nearly 100% (4). The WHO Global Task Force on XDR-TB has recommended timely access to drug susceptibility testing for all patients at risk for or suspected to have MDR/XDR-TB (9).

The microscopic observation drug susceptibility (MODS) assay is a relatively low-cost, simple liquid culture method that relies on microscopic detection of cording growth that is characteristic for *M. tuberculosis* (1,3,6,7,8). Potential advantages of MODS are relatively rapid mycobacterial growth in liquid media and reliance on microscopy skills similar to those used for smear microscopy. MODS has been reported to reliably identify *M. tuberculosis* isolates with resistance to isoniazid and/or rifampin (3,6,7,8).

We undertook a prospective study in two settings to further evaluate the performance of MODS for direct detection of *M. tuberculosis* drug resistance in pulmonary TB suspects at risk for drug resistance.

This study was performed as part of a larger study to evaluate performance of MODS for diagnosis of TB among pulmonary TB suspects at the National Thorax Institute in Honduras, and the Federal University of Rio de Janeiro in Brazil (1). Susceptibility testing was

performed for all TB suspects prospectively identified as having ≥1 of the following risk factors: a) suspected TB treatment failure, b) suspected TB relapse, c) treatment default, or d) close contact with an MDR-TB case (1). Informed consent was obtained from all subjects, and this study was approved by ethics committees all involved institutions.

Respiratory specimens submitted for routine care purposes were digested and decontaminated using the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method (5). Pellets were resuspended in a final volume of 2 ml and used immediately for inoculation of culture media.

Isoniazid stock solution (10,000 ug/ml) was prepared by dissolving 100 mg isoniazid (Sigma) in a final volume of 10 ml sterile distilled water. Rifampin stock solution (10,000 ug/ml) was prepared by dissolving 100 mg rifampin (Sigma) in DMSO, then adding sterile distilled water to a volume of 10 ml. Aliquots were frozen.

MODS liquid medium was prepared as previously described (8). Antibiotic stock solutions were diluted and added to MODS liquid medium to give the following critical concentrations: INH 0.1 μg/ml ("MODS/INH"), and RIF 2.0 μg/ml ("MODS/RIF"). For each respiratory specimen, 1 ml of drug-free MODS medium was dispensed into one well of a 24-well tissue culture plate (Costar, Corning, NY). One ml of MODS/INH medium was placed into each of 2 adjacent wells, and one ml of MODS/RIF medium was placed into each of 2 additional wells, for a total of 5 wells per respiratory specimen. 0.2 ml aliquots of decontaminated respiratory specimen were inoculated into each of the following: drug-free well, one MODS/INH well, and one MODS/RIF well. In addition, 0.2 ml aliquots of

decontaminated respiratory specimen diluted 1:10 in MODS medium were inoculated into each of the remaining MODS/INH and MODS/RIF wells. Specimens obtained from different subjects but processed on the same day were plated into different rows of the same plate, and the plate was placed within a gas permeable plastic bag. Plates were incubated at 37°C in 10% $CO_2$. The drug-free well was examined twice-weekly for growth and pellicle morphology for 8 weeks, using X40 inverted microscopy. A culture was considered positive for *M. tuberculosis* if microscopically observed bacterial pellicles had a corded appearance. Drug-containing wells were examined microscopically on the $14^{th}$ day after cording bacterial growth became visible in the corresponding drug-free well. For drug-containing wells, growth was recorded as positive (indicating drug resistance) if corded growth was visible, or negative (indicating susceptibility) if no corded growth was visible. The time interval of 14 days was chosen based on our previous work showing that susceptibility/resistance status in MODS was stable for at least two weeks after detection of growth (8), and in order to optimize efficiency by eliminating the need for daily microscopic examination of all drug-containing wells. The MODS technologist was not aware of LJ drug susceptibility test results.

LJ slants were prepared from commercially available powder medium base (Becton Dickinson), according to the manufacturer's instructions. Primary cultures were inoculated, examined, and interpreted as described (1). For susceptibility testing, several spadesful of growth were transferred from the primary LJ to a tube containing glass beads and sterile saline, and homogenized for 1 minute. After larger particles had been allowed to settle for 30 minutes, the supernatant was withdrawn, diluted to turbidity of a MacFarland No. 1 standard, and diluted to $10^{-3}$ and $10^{-5}$ in saline. For each dilution, 0.2 ml was inoculated onto each of

three LJ slants – one containing INH 0.2 ug/ml, one containing RIF 40 ug/ml, and one drug-free slant (2). Slants were incubated at 37ºC and colonies were enumerated on day 28. Drug resistance was defined as ≥ 1% or more growth of colonies on the drug-containing slant compared to the drug-free slant. The LJ technologist was not aware of MODS results.

Among 351 individuals at risk for drug-resistant TB, 180 (51.3%) were culture positive for *M. tuberculosis* by both MODS and LJ, and were therefore included in this analysis. By the comparator LJ testing method, 69 (38.3%) isolates were resistant to both isoniazid and rifampin, 23 (12.8%) were resistant to isoniazid alone, 5 (2.8%) were rifampin mono-resistant, and 83 (46.1%) were susceptible to both isoniazid and rifampin. Performance parameters of MODS are shown in Tables 1 and 2. MODS results are shown for undiluted MODS inocula, and for inocula diluted 1:10. MODS indicated as "resistant" some specimens that the comparator method indicated as "susceptible". This trend was slightly more marked when undiluted inocula were used for MODS. For isoniazid testing, specimen dilution resulted in correct reclassification of 4 specimens as susceptible. For rifampin testing, specimen dilution resulted in correct reclassification of 2 specimens as susceptible. Among 69 isolates classified as MDR by LJ testing, MODS (diluted or undiluted) correctly identified 66 (95.7%) as MDR. Median times to availability of susceptibility results were 21 days (interquartile range [IQR] 17-24) days for MODS and 49 days (IQR 46-55 days) for LJ ($p < 0.001$ by paired t-test).

A possible explanation for disagreement between MODS and the comparator LJ proportion method results lies in the qualitative nature of MODS. For MODS, unlike for solid agar methods, there are no discrete colonies to count, and therefore a resistance proportion cannot

be calculated. In MODS, any growth in drug-containing medium indicates drug resistance, whereas in solid agar testing, growth of less than 1% of that on drug-free medium is interpreted as susceptible. In addition, the bacillary burden in the inocula appeared to have a small effect on drug susceptibility testing, since level of agreement between MODS and the reference standard was slightly higher for 1:10 diluted inocula than for undiluted inocula. The impact on MODS susceptibility results of the timing of readings and inoculum bacillary burden warrants further study.

Our results are generally similar to those reported by Caviedes et al and initially by Moore et al (3,6). Caviedes et al. showed that MODS had approximately 90% concordance for rifampin susceptibility using a microwell alamar blue comparator assay. MODS (critical concentration 0.5 µg/ml) was sensitive for detection of resistance to rifampin, but, as in our study, MODS misclassified as resistant a number of strains that were classified as susceptible by the comparator method (3). In a retrospective study, Moore et al analyzed their database of results for 207 pretreatment respiratory specimens collected and cultured by MODS as a component of epidemiological studies in Peru (6). The comparator method was either an indirect microwell alamar blue comparator assay or tetrazolium microplate assay; MODS critical concentrations were isoniazid 0.4 µg/ml and rifampin 1.0 µg/ml. MODS sensitivity, specificity, PPV, and NPV were 81.1%, 96.9%, 85.7%, and 95.7% for isoniazid, and 100%, 98.3%, 57.1%, and 100% for rifampin. For both drugs, the low PPVs were a consequence of MODS indicating as resistant some isolates that were susceptible by the reference standard method. However, Moore et al. recently evaluated MODS performance for direct susceptibility testing using as reference methods indirect susceptibility testing by LJ or the automated MBBacT system, with the use of a microwell alamar blue assay for resolution of

discordant reference method results. For MODS, critical concentrations were 0.4 µg/ml for isoniazid and 1.0 µg/ml for rifampin. They reported 100% agreement for rifampin (338 isolates tested among which 10.7% were rifampin resistant by the reference method), and 96.7% agreement for isoniazid (334 isolates tested among which 19.5% were resistant) (7). Differences between results of various studies may in part be attributable to differences in MODS critical drug concentrations and comparator methods across studies. In addition, in our study, MODS drug-containing wells were examined microscopically on the 14$^{th}$ day after growth became visible in the corresponding drug free-well, whereas Caviedes and Moore recorded MODS susceptibility results on the same day that growth was observed in the drug-free well (3,6). In our study, delayed reading of the MODS wells could have contributed to misclassification of some strains as "resistant".

Taken together, available studies indicate that MODS used as a sole method for drug susceptibility testing could lead, in a small proportion of individuals, to inappropriate designation of isoniazid and/or rifampin resistance. However, the very low likelihood ratio of a negative test indicates that a negative MODS test (i.e. MODS test that is negative for resistance) is useful for "ruling out" resistance. This feature, and the relatively short time between specimen acquisition and susceptibility results, indicate that MODS might be useful as a direct drug susceptibility screening tool in order to prioritize *M. tuberculosis* isolates for subsequent indirect drug susceptibility testing using conventional methods. There is a need for standardization of MODS methodology for drug susceptibility testing.

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**AUTHORS' CONTRIBUTIONS**

Study design (Arias, Mello, Alvarado-Gálvez, Kritski, Fonseca, Chaisson, Kimerling, Dorman); key roles in study implementation and data collection (Arias, Mello, Pavón, Marsico, Alvarado-Gálvez, Rosales, Pérez, Andrade, Pessôa, Fonseca); data analysis (Arias, Mello, Kimerling, Dorman); manuscript writing (Arias, Mello, Chaisson, Kimerling, Dorman); manuscript review (all authors).

**CONFLICT OF INTEREST**

The corresponding author has had full access to all of the data in the study, and has had final responsibility for the decision to submit for publication. None of the authors has any financial or personal relationships with other people or organizations that could inappropriately influence their work.

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Table 1. Performance characteristics of MODS for detection of resistance to isoniazid.

| | | A) UNDILUTED MODS INOCULUM | | B) MODS INOCULUM DILUTED 1:10 | |
|---|---|---|---|---|---|
| | | **Reference Standard: LJ** | | **Reference Standard: LJ** | |
| | | **Resistant** | **Susceptible** | **Resistant** | **Susceptible** |
| **MODS** | **Resistant** | 89 | 19 | 89 | 15 |
| | **Susceptible** | 3 | 69 | 3 | 73 |
| Sensitivity % | (95% CI) | 96.7 | (92.1-98.8) | 96.7 | (92.2-98.8) |
| Specificity % | (95% CI) | 78.4 | (73.5-80.6) | 83.0 | (78.2-85.1) |
| PPV % | (95% CI) | 82.4 | (78.4-84.2) | 85.6 | (81.5-87.4) |
| NPV % | (95% CI) | 95.8 | (89.9-98.5) | 96.1 | (90.5-98.6) |
| (+) LR | (95% CI) | 4.48 | (3.48-5.10) | 5.68 | (4.22-6.65) |
| (-) LR | (95% CI) | 0.04 | (0.01-0.11) | 0.04 | (0.01-0.10) |
| Accuracy % | (95% CI) | 87.8 | (83.0-89.9) | 90.0 | (85.3-92.1) |

LJ, Lowenstein-Jensen; PPV, predictive value of a positive test; NPV, predictive value of a negative test; (+) LR, likelihood ratio of a positive test result, (-) LR, likelihood ratio of a negative test result.

Table 2. Performance characteristics of MODS for detection of resistance to rifampin.

| | | A) UNDILUTED MODS INOCULUM | | B) MODS INOCULUM DILUTED 1:10 | |
|---|---|---|---|---|---|
| | | **Reference Standard: LJ** | | **Reference Standard: LJ** | |
| | | **Resistant** | **Susceptible** | **Resistant** | **Susceptible** |
| **MODS** | **Resistant** | 72 | 18 | 72 | 16 |
| | **Susceptible** | 3 | 87 | 3 | 89 |
| Sensitivity % (95% CI) | | 96.0 | (90.3-98.6) | 96.0 | (90.4-98.6) |
| Specificity % (95% CI) | | 82.9 | (78.8-84.7) | 84.8 | (80.7-86.6) |
| PPV % (95% CI) | | 80.0 | (75.2-82.1) | 81.8 | (77.0-84.0) |
| NPV % (95% CI) | | 96.7 | (91.9-98.8) | 96.7 | (92.1-98.8) |
| (+) LR (95% CI) | | 5.60 | (4.25-6.44) | 6.30 | (4.69-7.36) |
| (-) LR (95% CI) | | 0.05 | (0.02-0.12) | 0.05 | (0.02-0.12) |
| Accuracy % (95% CI) | | 88.3 | (83.6-90.5) | 89.4 | (84.7-91.6) |

LJ, Lowenstein-Jensen; PPV, predictive value of a positive test; NPV, predictive value of a negative test;

(+) LR, likelihood ratio of a positive test result; (-) LR, likelihood ratio of a negative test result