Training Laboratory Technicians from the Ethiopian Periphery in the MODS Technique Enables Rapid and Low-Cost Diagnosis of *Mycobacterium tuberculosis* Infection

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Abstract. Tuberculosis (TB) is a leading cause of morbidity and mortality and is frequently complicated by emergence of drug-resistant strains. Diagnosis of TB in developing countries is often based on the relatively insensitive acid-fast staining that does not enable susceptibility profiling. Microscopic observation drug susceptibility assay (MODS) is an inexpensive, simple method that enables rapid TB culture coupled with susceptibility testing. A 3-week MODS training of three Ethiopian laboratory technicians was conducted at Hadassah-Hebrew University Medical Center, Israel. Results of the trainee readings were blindly assessed by an experienced instructor. Two hundred fifty-five (255) trainee culture readings were evaluated throughout the course. The sensitivity and specificity were 75–100% and 31.5–100%, respectively. Multivariate analysis revealed that sensitivity and duration of incubation were positively correlated, although specificity was positively correlated with the length of training. MODS can be reliably performed by laboratory technicians inexperienced in culture techniques in developing countries, with high sensitivity and specificity reached after a brief learning period.

INTRODUCTION

Mycobacterium tuberculosis is the second leading infectious cause of death worldwide, resulting in death of more than 1.5 million people annually, despite effective and available treatment.^{1–3} Although the majority of tuberculosis (TB) patients in developed countries are readily diagnosed and treated, many TB patients in developing countries, including sub-Saharan Africa, the Far East, South America, and the former Soviet republics, are under-diagnosed and poorly treated.^{4,5}

Several factors account for this widespread treatment and diagnostic failure. One such factor is the human immunodeficiency virus (HIV) epidemic, which is neither controlled nor treated in many developing countries, and contributes to emergence of TB in acquired immunodeficiency syndrome (AIDS) patients.⁶ Another major factor is the lack of cheap, easy-to-use, and sensitive methods for diagnosis of TB and anti-TB drug susceptibility in remote rural areas.⁷ A third factor, not limited to developing countries only, is the emergence of multidrug-resistant TB and more recently of extensively drug-resistant-TB (XDR-TB).^{8–12}

Acid-fast staining of sputum, still considered as the main method used for the diagnosis of pulmonary TB in developing countries, is insensitive, and positive only when the density of bacilli is 10,000 or more per milliliter (mL) of sputum.¹³ Consequently, this method fails to identify about 25–50% of the patients with TB,^{14–17} and is even less sensitive in HIV-positive patients (9–57%).^{17,18} More sensitive methods for diagnosis of TB are polymerase chain reaction (PCR) of the sputum, cultures on solid media (Lowenstein-Jensen or Middlebrook formulations), and liquid media.¹³ The main disadvantages of these methods are their high cost (e.g., commercial PCR systems and liquid culture in automated systems)

and the long duration of incubation needed to obtain positive culture and drug susceptibility results (culture on solid media), which make these techniques unsuitable for use in remote third world regions.^{13,19} A new automated assay that provides a rapid molecular detection of TB as well as resistance profile to rifampin in 90 minutes was recently published. This assay although rapid and simple is still too expensive, and requires infrastructure that is not widely available in developing countries.²⁰

Microscopic observation drug susceptibility assay (MODS), is a new laboratory technique that was developed by researchers from Johns Hopkins in collaboration with researchers from Universidad Peruana Cayetano Heredia and AB PRISMA in Lima, Peru.^{21–24} This inexpensive and low tech method enables rapid culture of *M. tuberculosis* and concomitantly provides a reliable sensitivity profile of isolated bacteria to the anti-TB medications isoniazid and rifampin.^{25,26} These advantages make this approach an attractive new option for TB diagnosis in developing countries. Recently, the World Health Organization (WHO) endorsed MODS as an interim solution in resource-limited settings.^{27,28} Important questions remain to be answered before the use of MODS can be scaled up. Major issues that should be addressed are the full cost implications, whether direct or indirect inoculation should be used, accurate specificity assessment of the method, ways to reduce the contamination rate, and standardization of the technique, including the isoniazid concentration that should be used. Another important concern is the ability to train laboratory technicians outside of research settings in developing countries who are inexperienced in the use of culture techniques (especially for TB) to validly perform MODS.²⁷ A training program in the use of MODS by Ethiopian laboratory technicians was conducted in 2006 at the Tuberculosis Laboratory, Hadassah-Hebrew University Medical Center in Israel, and offered an opportunity to test the feasibility of the use of this approach by laboratory personnel from resource-limited areas. We report the results of this training and the performance of the trainees in the use of MODS to accurately diagnose TB and anti-TB drug susceptibility.

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METHODS

Participants. During June 2006, a 3-week training program was conducted at the TB laboratory in Hadassah-Hebrew University Medical Center in Jerusalem. The trainees were one Israeli technician and three Ethiopian laboratory technicians, two from TB laboratories in Ethiopia's smaller cities where no culture technique was available, and one from the Central TB reference laboratory in Addis Ababa. The master instructor was one of the developers of the MODS technique (LC).

The program included theoretical lectures and hands-on experience with the technique, using 52 sputum samples from pulmonary TB patients seen at the Addis Ababa St. Peter's TB hospital, some of whom were treated and some were untreated. Aliquots of these specimens were brought to Israel for training purposes.

TB cultures. Cultures were performed according to previously published MODS protocols.²⁰ In brief, sputum samples were decontaminated using NaOH and liquefied by N-Acetyl-Cysteine for 15 minutes, dissolved with phosphate buffered saline (PBS) pH 6.8 0.067M, and the mixture was centrifuged for 15 minutes at 3,000 rpm. The pellet was dispersed using liquid Middlebrook broth (7H9) oleic acid, albumin, dextrose, and catalase (OADC) (Becton Dickinson) and a mixture of antibiotics was added, including polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin. The 720 μ L of the final mixture was inoculated in six wells of a 24-well plate (Becton Dickinson) as follows: two drug-free wells, two wells containing 0.4 μ g/mL isoniazid, and two wells containing 1.0 μ g/mL rifampin. The plates were inserted into plastic bags, sealed, and incubated at 37°C for an average of 12.4 days (range 9–15). Starting on the fifth incubation day, plates were observed under an inverted light microscope. Each plate was examined by a trainee and by the master trainer, and the trainee's reading was recorded. The trainee read the plate before the trainer, and all trainees were blinded to the readings both of the trainer and the other trainees.

A culture was considered positive when at least two colonies (appearing as cords) were observed in at least one of the two control (drug-free) wells. Isoniazid-resistant and rifampin-resistant TB was defined by the appearance of cords in at least one control well and in both wells containing the same drug (isoniazid or rifampin, respectively). When colonies were identified in only one of the same drug wells, the result of the resistance test for that drug was defined as indeterminate. Multidrug resistant TB was considered when the cords appeared in control wells and in all four wells containing isoniazid and rifampin. For each sample, time (incubation days) to positive results and the sensitivity to anti-TB drugs were recorded.

Evaluation of trainees' performance. During the course the trainees prepared and observed the cultures under guidance of the master instructor. The instructor inspected the cultures every day and recorded her results, without knowing the trainees' observations. The results were used as a reference for evaluation of the trainees' performance and for personal feedback to each of the trainees at the end of each training day. Day of training (i.e., trainee "experience") was defined for each individual trainee as his or her cumulative days of plate reading. To expand the number of plates read by each trainee and to expose the trainee to plates at different incubation periods, some of the plates were read by the same trainee

on different days. Because we could not de-identify the samples, repeating plates were always presented in combination with other plates to decrease the chance of their being recognized by the trainees. The trainees were blinded to the acid fast staining results of each of the samples as well as their own previous readings of the plates.

Statistical analysis. For each trainee the sensitivity and specificity of his or her reading of the control wells were calculated every day. On each day the results of all the trainees were evaluated by the same parameters. Although there were officially four trainees in the course, one of the technicians did not participate regularly in the course and the results of his readings were not included in the analysis of the training performance. Univariable and multivariable analyses were performed to estimate the impact of the training duration, incubation time, and the different trainees upon the accuracy of the trainees' readings. These were carried out by logistic regression models, with two dummy variables for the three trainees.²⁹ The results were presented in terms of odds ratios (OR), based on the odds of successfully identifying a true positive (sensitivity) or a true negative (specificity) culture, using the master instructor's readings as the reference. Confidence intervals (CI) (95%) were calculated as well. *P* values of 5% or less were considered statistically significant.

RESULTS

Rate of positive cultures. Fifty-two sputum samples originating from Ethiopia were used during the training. According to acid-fast staining performed in Ethiopia, 47 of 52 samples used were acid-fast positive, whereas five were acid-fast negative. To expand the number of training samples, most of the samples were cultured more than once. Nineteen of the sputum samples were cultured once, 23 of 52 twice, 8 of 52 were cultured in triplicates and two samples were cultured in quadruplicate. Altogether, 97 MODS cultures were performed during the study period. Samples from 42 (80.7%) patients were positive in at least one culture, although one sample was contaminated and nine were always negative. When relating to each culture independently, 71 of 97 were positive (73%), 23 were negative (23%), and 3 were contaminated (3%). In six cases there was inconsistency in the results of the same sample that was processed more than once. Mean time to positive results of MODS cultures was 7.5 days, and 90.1% of the positive results became positive by Day 10 (Figure 1).

Anti-TB drug resistance profile. Thirty (71.4%) of the 42 positive samples were sensitive to both isoniazid and rifampin, 5 (11.9%) were resistant to isoniazid only, 1 (2.4%) was resistant to rifampin only, whereas 2 samples (4.76%) yielded indeterminate drug sensitivity results caused by discrepancy between the results of the two wells containing the same drug. In 3 samples (7.1%) there was false multidrug resistance that originated from a processing error—no drugs were added to the drug wells.

Trainee performance. Trainees 1, 2, and 3, respectively, read 98, 66, and 91 individual plates over 7, 5, and 5 training days. We evaluated the sensitivity and specificity of the trainee readings of the control wells (total of 255 readings) and the drug susceptibility wells during every training day (total readings of isoniazid and rifampin were 155 each). The sensitivity of reading control wells was high during the entire training period, ranging from 75% (Day 4) to 100% (Days 2

FIGURE 1. Time from incubation of sputum culture by microscopic observation drug susceptibility assay (MODS) to positive results. Ninety-seven (97) samples were incubated, 71 (73%) were positive.

and 7) (Figure 2). The specificity of control culture readings ranged from 31.5% (Day 1 of culture reading) to 100% (Days 2, 3, 4, 6, and 7) (Figure 3). Over the entire training period, the sensitivity and specificity, respectively, of TB diagnosis for the three trainees ranged from 89% to 100% and 55% to 97%; and for drug susceptibility readings ranged from 67% to 100% and 71% to 98% (Table 1).

Multivariable logistic regression analysis revealed a positive relationship between the duration of incubation and sensitivity (OR = 1.96, 95% CI = 1.06–3.64), and between the day of training and specificity of TB diagnosis (OR = 2.11, 95% CI = 1.13–3.97).

Investigating the increase in specificity with more experience, we noted that it resulted mainly from the low specificity (13%) of trainee 3's readings on his training Day 1, and his subsequent improvement (Figure 4). On his first training day, trainee 3 read many more negative plates than the other trainees (15 versus 1 and 3), with 7 of 15 incubated for only 5 days and 8 of 15 incubated for 6 days. The two other trainees read fewer and somewhat more mature negative plates on

their first training day—one plate of 6 days incubation for trainee 1, and two and one plates, respectively, of 6 and 7 days incubation for trainee 2—and both had 100% specificity. Nevertheless, by his fourth training day, trainee 3 correctly read 3 of 3 negative plates of 5 days incubation (Figure 5D), and all trainees maintained 75% or greater specificity after that (Figure 4), in accordance with the positive relationship between trainee experience and specificity shown by the regression analysis.

Figure 5 underscores the regression finding of a positive relationship between sensitivity and incubation duration, showing that for both trainees 1 and 3, sensitivity dropped on training Day 4 when reading plates of 5 days incubation.

Multivariate logistic regression analysis of the sensitivity of the trainee readings of drug susceptibility did not show any difference in performance with increased incubation (OR = 0.88, 95% CI = 0.43–1.81, $P = 0.73$). The specificity of drug susceptibility readings was influenced by the specific trainee, was higher with rifampin than isoniazid-containing wells (OR = 5.71, 95% CI = 1.77–18.52, $P = 0.004$), and

FIGURE 2. Sensitivity of control well readings, by training day. The n = number of reference standard positive plates (true positive + false negative) read by all trainees on each day.

FIGURE 3. Specificity of control well readings, by training day. The n = number of reference standard negative plates (true negative + false positive) read by all trainees on each day.

increased with training experience (OR = 1.49, 95% CI = 1.04–2.15, P = 0.030).

DISCUSSION

Mycobacterium tuberculosis is still a major health problem in developing countries, caused by its increasing prevalence and the frequent unavailability of cheap, sensitive, and specific diagnostic tools. The method most commonly used to identify TB infection is acid-fast staining of sputum, body fluids, and tissues, which features only a 50% sensitivity rate.¹³ Culture for TB, performed only in central laboratories in major cities, is a luxury that most patients in developing countries cannot afford, moreover susceptibility testing to anti-TB drugs is rarely available. This paucity of quick and affordable diagnostic tools

results in under-diagnosis of TB infection in many patients, as well as erroneous, often toxic prolonged treatment in others.^{6,9}

MODS, Microscopic Observation Drug Susceptibility assay, is a novel method for the diagnosis of TB infection and determination of susceptibility to anti-TB medications. This technique has been previously validated and shown to be quick and reliable^{23–26} and has been implemented in the regional laboratories of the national TB program in Lima-Peru,^{30,31} but otherwise has not been tested in the “field,” in regional or district laboratories of low resource settings, for which it is intended. Demonstrating the ability to train laboratory technicians outside of research settings in developing countries to validly perform MODS, has been identified as a research priority.²⁷ In this study, we found MODS to be highly suitable for use by laboratory technicians working in TB laboratories where no culture technique is available and using sputum specimens from patients of the same region. The technicians reached high levels of technical competence in the application of MODS after only a 3-week training period, although some differences in the ability of the trainees were noticed.

The major parameter that was found to improve during the course of the training was the specificity of the trainee readings of the culture wells. This improvement was largely the result of the low specificity for one trainee during his first day caused by the combination of his lack of experience and the short incubation duration of the plates he read. By his fourth training day, this trainee was successfully analyzing plates incubated for as short as 5 days with high specificity, and all the trainees maintained 75% or greater specificity by their fifth day of training.

Sensitivity appeared to be more markedly influenced by the incubation age of the plates. The fact that we could not show statistically that the training itself improved the sensitivity of the readings may be related to the close correlation of incubation and training time and in the increase in sensitivity with longer incubation. Sensitivity was high from the start, and the slight decline in sensitivity on the third and fourth days of the course may have been caused by more slides of low incubation age being read on those days. The results subsequently improved to 100% on the seventh and final day of training.

The technical conclusion from this training course is that training in the MODS technique should be started with plates of

TABLE 1

Trainees' overall performance in tuberculosis (TB) diagnosis and drug susceptibility reading during the course

TB diagnosis				
	N positive	Sensitivity	N negative	Specificity
Trainee 1	66	0.89	32	0.97
Trainee 2	35	1	31	0.94
Trainee 3	60	0.93	31	0.55
Combined	161	0.93	94	0.82
INH susceptibility				
Trainee 1	10	0.9	43	0.93
Trainee 2	4	1	28	0.71
Trainee 3	5	1	65	0.92
Combined	19	0.95	136	0.88
Rifampin susceptibility				
Trainee 1	5	0.8	52	0.98
Trainee 2	0	–	29	0.9
Trainee 3	1	0	68	1
Combined	6	0.67	149	0.97
INH + Rifampin susceptibility				
Trainee 1	15	0.87	95	0.96
Trainee 2	4	1	57	0.81
Trainee 3	6	0.83	133	0.96
Combined	25	0.88	285	0.93

FIGURE 4. Specificity of control well readings, for each trainee by training day (experience day). The n = number of reference standard negative plates (true negative + false positive) read by each trainee.

sufficient age, with younger, more challenging plates gradually being introduced as the trainees' readings skills increase over the course of the training. Deriving from prior experience of the trainer, the earliest the slides can be accurately read by an experienced microscopist is about 5 days. During the incubation, cords of TB become more prominent, making identification of positive results easier (Figure 6). For inexperienced

trainees, specimens should be prepared a few days in advance of the training course so that plates of sufficient age will be available at the start of training. Because the trainees also spend some days initially learning how to prepare the specimens, the pre-course preparation should be timed so that plates of 7–8 days old are available on the first day that they would be expected to read plates.

FIGURE 5. Influence of incubation time and training day on sensitivity and specificity of TB diagnosis by trainees 1 and 3. For each trainee (1 and 3), two graphs were plotted, describing, respectively, the sensitivity and specificity of TB diagnosis on each day of training, stratified by the incubation day (inc. day), specified in different colors. The numbers adjacent to the line specify the number of reference standard plates.

FIGURE 6. Microscopic observation drug susceptibility assay (MODS) positive culture well at different incubation times, objective $\times 100$. (A) 5 days, (B) 7 days, (C) 9 days, and (D) 15 days.

Our results show the ease with which MODS can be learned reaching high sensitivity and specificity. In addition to its diagnostic validity, the use of MODS carries several additional advantages pertinent to remote areas in developing countries. Utilization of MODS requires neither expensive equipment nor prepared special reagents.^{22–24} The use of simple and available reagents addresses the problem of delivery of prepared supplied reagents to remote areas with a final cost for culture and susceptibility for one specimen of $\sim 2\text{--}3$ US\$.²¹

We obtained positive culture results in 5–10 days (Figure 1). The median time to culture positivity reported in larger studies was 7–9 days^{21,23}; this would enable local physicians to wait for culture results rather than starting empiric anti-TB treatment. In addition, because multidrug resistant TB is an emerging health problem in developing countries, MODS enables the tailoring of suitable anti-TB drug regimens according to reliable susceptibility result with no delay and without additional cost.

Our study has several limitations. First, most of the examined sputum specimens were acid-fast positive. In sputum samples with less clear and slower growth rates the sensitivity and specificity rates may be lower than observed in this study. Second, the fact that the number and character of the plates read by the trainees was not identical on each day resulted in some potential variation in their learning curves. Another limit in the training design was the reading of some plates more than once by the same trainee without de-identifying them. Use of the same plates on different training days enabled each trainee to be exposed to a blend of plates at different incubation ages on the same day. We could not de-identify the plates for logistic reasons, but we tried to overcome this problem by always presenting repeating plates in combination with

other plates. Although the probability of identifying a plate when re-encountered and recalling the previous reading result is low, we cannot exclude this completely. We suggest that during future training courses, plates that are to be reused should be recoded.

In conclusion, our study clearly shows that utilization of the MODS method by inexperienced technicians in culture techniques is feasible and effective, with high degrees of sensitivity and specificity reached after only a short training period. The use of MODS is highly reliable in settings where rapid and affordable diagnosis is essential. Further training of local personnel from developing countries in the use of this technique may lead to better diagnosis and treatment strategies for TB infection in remote area of developing countries, where accurate TB diagnosis is mostly needed.

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