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Rapid Diagnosis of Tuberculosis and Multidrug Resistance by the Microscopic-Observation Drug-Susceptibility Assay

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Running Head: Rapid TB & MDR Diagnosis by MODS assay in HIV setting

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject: TB is the leading cause of mortality in HIV-infected persons and is frequently associated with a delay in diagnosis, in part due to limitations of currently available tests, particularly in high HIV prevalence settings. The microscopic-observation drug-susceptibility (MODS) assay is a simple, rapid, low-cost method for diagnosis of TB and multidrug-resistance. However, no studies have been performed in sub-Saharan African settings with a high prevalence of TB/HIV co-infection and multidrug resistance.

What the Study Adds to the Field: This study measured the performance of the MODS assay in a cohort of predominantly HIV-infected TB suspects from South Africa and found that: (1) MODS detected *M.tuberculosis* with high sensitivity and greater speed compared to both agar and MGIT liquid culture methods, and (2) MODS provided rapid and reliable results for diagnosis and exclusion of MDR-TB. These findings are consistent with previous findings from low-HIV-prevalence settings and provide support for expanding MODS use to similar settings in sub-Saharan Africa.

ABSTRACT

Rationale: Mortality is exceedingly high and rapid among HIV-infected tuberculosis (TB) patients, in part due to limited access to appropriate TB diagnostics. The microscopic observation drug-susceptibility (MODS) assay is a simple, rapid, low-cost test for TB and multidrug-resistant (MDR) TB, but data in HIV-infected individuals and in Africa are limited.

Objectives: To evaluate the MODS assay in a high-HIV-prevalence setting

Methods: We performed a prospective diagnostic accuracy study of consecutive adult TB suspects from outpatient and inpatient settings at a district hospital in rural South Africa. Sputum was tested by concentrated smear microscopy, agar (Middlebrook 7H11) and liquid (MGIT) culture, and the MODS assay. Drug-susceptibility testing (DST) was by indirect 1% proportion method and MODS. Reference standard for *M.tuberculosis* detection was growth on Middlebrook or MGIT culture; 1% proportion was the reference standard for isoniazid and rifampin DST.

Measurements and Main Results: Among 534 TB suspects enrolled, 388 (73%) were HIV-positive, with a median CD4 count of 161 cells/mm^3 (IQR: 72–307). TB was diagnosed by the reference standard culture in 113 (21%). MODS sensitivity was 85% (95% CI: 78–92%), while specificity was 97% (CI: 95–99%). MODS test performance did not differ by patients' HIV status (sensitivity 88% vs 90%; specificity 97% vs 100% for HIV-positive vs HIV-negative, respectively). For MDR-TB diagnosis (n=11), sensitivity was 100% (1-sided CI: 68-100%) and specificity, 94% (CI: 82–98%). Median TAT for MDR-TB diagnosis was 7 days (IQR: 6–9) with MODS vs. 70 days (IQR: 49–96) with indirect proportion method ($p<0.001$).

Conclusions: Among predominantly HIV-infected TB suspects, MODS provided high sensitivity and specificity for rapid diagnosis of TB and MDR-TB. Given the high mortality from

TB and MDR-TB and prolonged opportunity for TB transmission before diagnosis, the MODS assay warrants serious consideration for use in similar high HIV prevalence, resource-limited settings.

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INTRODUCTION

Tuberculosis (TB) is a leading cause of morbidity and mortality among HIV-infected patients worldwide. In sub-Saharan Africa, the TB and HIV epidemics are closely intertwined, with more than 70% of all TB cases in South Africa co-infected with HIV.(1,2) Diagnosing TB in HIV-infected patients is challenging because of not only the atypical clinical presentation of TB disease, but also the paucibacillary nature of pulmonary TB disease in HIV patients. The most widely-used TB diagnostic test worldwide is sputum smear microscopy, which fails to detect TB in over 60% of cases, particularly in high HIV prevalence settings.(3-6) Smear-negative TB in HIV-infected persons is associated with poorer outcomes, in part due to delays in TB diagnosis and treatment initiation.(7,8) Although mycobacterial culture can provide added diagnostic sensitivity, the vast majority of TB suspects lack access to this, given the need for sophisticated and expensive laboratories. There is an urgent need for simple, rapid, affordable diagnostic tests, more sensitive than smear microscopy, which can be used for TB diagnosis in resource-limited, high HIV prevalence settings.(9,10)

In addition to the rapid rise in drug-susceptible TB incidence in sub-Saharan Africa, mulitdrug-resistant (MDR) TB has recently emerged as a growing cause of mortality among TB/HIV co-infected patients.(11,12) Diagnosis of MDR-TB requires microbiologic evaluation of the *M.tuberculosis* isolate's drug-susceptibility, which is not possible with smear microscopy and, instead, requires mycobacterial culture. Of limited availability in many areas most burdened by MDR-TB, current techniques for DST are further complicated by turnaround times of 6-8 weeks. This long delay results in disease progression, and often death among HIV co-infected patients,(13,14) in addition to ongoing transmission of MDR-TB strains in healthcare and community settings. A better diagnostic test for MDR-TB that has a faster turnaround-time, and

that can be implemented in peripheral healthcare settings, affording accessibilty to a substantially larger proportion of the population is needed.

In recent years, several rapid assays for TB diagnosis have been developed.(15,16) The microscopic-observation drug-susceptibility (MODS) assay is a simple, rapid, low-cost method which holds great promise for resource-limited settings.(17-21) MODS detects TB and drug resistance directly from sputum using liquid broth media, and has been found to be highly-sensitive and specific in rapidly detecting TB and MDR-TB compared with conventional liquid culture. However, these studies have not been performed in sub-Saharan African settings with a high prevalence of TB/HIV co-infection.

South Africa has the largest HIV burden worldwide,(22) and also among the highest TB incidence (948 cases per 100,000 population).(23) Moreover, MDR-TB has recently emerged as a widespread epidemic.(24,25) In the province of KwaZulu-Natal (KZN), there were over 100,000 cases of TB, including 3000 MDR-TB cases reported in 2007 (30 cases per 100,000 population).(13) Eighty percent of all TB cases and 90% of MDR-TB cases were HIV co-infected. Although mycobacterial culture and DST are available, they are only performed at a single central laboratory in KwaZulu-Natal and take 6-12 weeks for results. Moreover, the vast majority of TB suspects continue to be evaluated by smear microscopy alone, due to current policies that limit culture and DST only to high-risk patients (i.e, treatment failures and re-treatment cases). Thus, we evaluated the MODS assay to determine its performance in diagnosis of TB and MDR-TB in a high HIV-prevalence setting. Some of the results of these studies have been previously reported in the form of an abstract.(26)

METHODS

Study population

Consecutive adult TB suspects were enrolled from inpatient and outpatient settings at a district hospital from June 2008 through April 2009. Study staff actively screened adults for the presence of either cough (any duration) or ≥2 other TB symptoms (fever, night sweats, weight loss, chest pain, or shortness of breath of any duration). Patients were eligible if they reported symptoms and were new TB suspects (i.e., not currently taking anti-tuberculosis medications) or treatment failures (i.e., receiving anti-TB medications for ≥2 months, but with recurrence or persistence of TB symptoms).

Sample collection

All subjects submitted a single "spot" sputum sample. Patients diagnosed with TB were offered HIV testing, as per current routine practice at the district hospital. CD4 cell count was obtained on all known HIV-positive patients, either at the time of enrollment or abstracted from the medical chart within one month before or after enrollment.

Laboratory methods

Sputum specimens were transported to the TB research laboratory at the Nelson R Mandela School of Medicine, University of KwaZulu-Natal in Durban for standard culture, DST, and MODS testing within 48 hours of collection. All samples were stored at 4° C prior to and during transport to the laboratory.

M.tuberculosis detection

The methods for sputum decontamination, culture, and drug-susceptibility testing in this setting have been previously described (27) and are available in the online appendix. Briefly, sputum samples were digested using the N-acetyl-L-cysteine (NALC)–sodium hydroxide (NaOH) method. The re-suspended sediment was divided for parallel testing by MODS and two standard culture techniques: Middlebrook 7H10 agar plates and BACTEC^{TM} MGIT^{TM} 960 broth (Becton Dickinson, Sparks, MD, USA).

Middlebrook agar plates were read at three weeks and six weeks for *M.tuberculosis* growth. MGIT broth tubes were continuously monitored for 42 days for *M.tuberculosis* growth. MGIT cultures that were contaminated prior to 42 days were re-decontaminated and re-cultured. All positive cultures by MGIT were identified as *M. tuberculosis complex* by niacin and nitrate reductase tests.

The MODS assay was performed in accordance with published standard operating procedures,(28) with minor variations noted in the online appendix. For each patient sample, 4 wells were used: 2 drug-free wells, 1 with isoniazid at 0.4 μg/ml, and 1 with rifampicin at 1 μg/ml. MODS cultures were examined using an inverted light microscope at 40x magnification every day from day 4 through day 21. Positive MODS cultures were identified by presence of characteristic cord formation in the drug-free control wells.

Drug-susceptibility testing

Indirect drug-susceptibility testing (DST) was performed on all positive isolates from the standard culture using the 1% proportion method on Middlebrook 7H10 agar to isoniazid (1.0 ug/mL), rifampicin (1.0ug/mL), ethambutol (7.5ug/mL), and streptomycin (2.0ug/mL).

Direct drug-susceptibility testing was performed with the MODS assay for isoniazid (0.4 ug/mL) and rifampicin (1.0ug/mL). Growth in drug-free control wells but not in drug-containing wells indicated a fully-susceptible strain; growth in drug-free and in a drug-containing well indicated resistance. Drug-sensitive and multidrug-resistant control strains were included on each MODS plate. A subset of MODS cultures did not undergo drug-susceptibility testing, and therefore, only 60 specimens had concurrent MODS isoniazid and rifampin wells for comparison to the 1% proportion method.

Definitions and Outcome Measures

A positive reference result was defined as a positive culture on either Middlebrook or MGIT culture.

The primary outcome measures were sensitivity, specificity, positive predictive value, negative predictive value, and turnaround time of the MODS assay compared to standard reference methods for: 1) detection of *M.tuberculosis*, and 2) diagnosis of drug-resistant TB. Turnaround time (TAT) was defined as the time from specimen processing to the time of culture and, if culture-positive, DST result. Secondary outcomes included performance of MODS, stratified by HIV status and sputum smear AFB result.

Statistical analysis

We calculated simple proportions and 95% confidence intervals (CI) for all analyses of sensitivity, specificity, and predictive value. For categorical variables, we compared proportions using chi-square tests and Fisher's exact test. For continuous variables, we compared medians using the Wilcoxon rank-sum Test. Turnaround time (TAT) was determined using survival

analysis techniques and compared using the log-rank test. Samples that were positive by MODS and both reference standard methods were included in this head-to-head analysis of TAT. A two-sided p-value of <0.05 was considered significant.

Data were analyzed using SAS, software version 9 (Cary, NC, USA).

Ethical Considerations

This study was reviewed and approved by the institutional review boards at Albert Einstein College of Medicine, Yale University, and the University of KwaZulu-Natal, and by the KwaZulu-Natal Department of Health.

RESULTS

Patients and samples

We collected sputum samples from 534 consecutive adult TB suspects, of whom 354 (66%) were female and median age was 38 years (interquartile range [IQR]: 31–48; **Table 1**). There were 475 (89%) TB suspects with no prior TB history and 59 (11%) who were currently failing to respond to first-line TB treatment. Among persons with known HIV status, 388 (87%) were HIV-positive and the median CD4 cell count was 161 cells / mm^3 (IQR: 72–307).

Among 534 TB suspects enrolled, 113 (21%) were identified with TB from either solid or liquid culture (**Table 1**). Of these, 63 (56%) were smear-positive and 50 (44%) were smear-negative. Among smear-positive samples, 19 (30%) were graded as scanty or 1+ and 44 (70%) were 2+ or 3+.

Sensitivity and specificity of TB detection

Overall sensitivity of MODS was 85% (95% confidence interval [CI]: 78–92%), while specificity was 97% (CI: 95–99%), when compared to the reference standard of solid or automated liquid culture (**Table 2)**. MODS sensitivity differed by AFB smear status, such that sensitivity was 95% (CI: 90–100%) among smear-positive TB cases and 72% (CI: 60–84%) among smear-negative TB cases. Negative predictive value for excluding TB among smear-negative TB suspects was 97% (CI: 94–99%).

Sensitivity did not differ between HIV-positive and HIV-negative patients (88% vs 90%, respectively; p=1.00), nor did specificity (97% vs. 100%, respectively; p=0.37). Negative predictive value for exclusion of TB was high in both groups (96% in HIV-positive, 98% in HIV-negative).

Among the 17 TB cases that were not detected by the MODS assay (i.e., false-negative results), 14 (82%) were smear-negative. Among 13 MODS false-positive results (i.e., reference standard-negative), repeat culture from the original specimen was negative, confirming these 13 as false-positive MODS results. No MODS cultures were contaminated by bacterial or fungal growth.

Direct drug-susceptibility testing for MDR-TB

A subset of MODS cultures did not undergo drug-susceptibility testing, and therefore, only 60 specimens had concurrent MODS isoniazid and rifampin wells for comparison to the 1% proportion method. Among these samples, resistance to isoniazid was detected in 13 (21%), to rifampin in 14 (23%), and to both isoniazid and rifampin (i.e., MDR-TB) in 11 (18%) by the reference standard (**Table 3**). Sensitivity of the MODS assay for detection of resistance to

isoniazid, rifampin, and MDR-TB was 100%; specificity was 92% (CI: 85–99%), 87% (CI: 78–96%), and 93% (85–99%), respectively.

Time to detection of *M.tuberculosis* and drug resistance

Of the 113 sputum samples positive for *M. tuberculosis* by the reference standard, 96 were culture-positive according to all three culture methods and were thus included in the head-to-head analysis of time to culture positivity (**Figure 1a**). The median time to culture positivity was significantly shorter for MODS than for the automated MGIT liquid or Middlebrook agar cultures (MODS 9 days [IQR 6-12] vs. MGIT 16 days [IQR 12-48] vs. Middlebrook 29 days [IQR 20-41], $p<0.001$ for all pairwise comparisons). Smear status had a significant effect on time to culture positivity by MODS (median: 7 days for a smear-positive vs. 12 days for a smear negative; $p<0.001$).

Turnaround time for diagnosis of MDR-TB was 7 days (IQR 6–9) for MODS as compared to 70 days (IQR 49–96) with the proportion method ($p<0.001$, **Figure 1b**).

DISCUSSION

This study evaluated the performance of the MODS assay in a high HIV prevalence setting and provides support for expanding its use to similar settings in sub-Saharan Africa where the TB and HIV epidemics are closely linked. MODS detected *M.tuberculosis* with high sensitivity and greater speed compared to both agar and MGIT liquid culture methods among HIV-infected TB suspects. The MODS assay is unique in that it offers a low-cost, simple, culture-based approach to diagnosing TB disease. Moreover, MODS provided rapid and reliable

results for diagnosis and exclusion of MDR-TB. These findings are consistent with previous findings from low-HIV-prevalence settings and are particularly notable in an era of growing HIV and MDR-TB worldwide.(29)

In addition to offering advantages over standard culture methods with respect to turnaround time, MODS provided substantial improvements beyond the current most widely-used TB diagnostic test, smear microscopy. For smear-positive patients who would be diagnosed by microscopy, MODS can augment smear by providing rapid results of drug-susceptibility testing. Among smear-negative patients, MODS can provide additional case detection and rule-out TB accurately. This study illustrates this in that 44% of confirmed TB cases had smear-negative TB disease that, according to most National TB Program guidelines, may have required additional tests such as chest radiography, antibiotic trial, and additional sputum smears before a diagnosis of TB could be made or excluded. Thus, although the sensitivity was lower among patients with smear-negative TB disease, the patients detected by MODS represent cases that would have been otherwise missed by smear and in whom diagnosis would have been delayed or never made. While implementation of MODS in facilities currently performing only smear would require technical and physical upgrading of facilities, training can be completed in a short time and the non-proprietary nature of MODS substantially limits costs.

Our findings in HIV-positive patients, who are known to have high rates of smear-negative TB, are of critical public health and individual patient-level importance. MODS test performance did not differ by HIV status of the patients, indicating that this test should be considered for use in all TB suspects presenting to care and treatment facilities, rather than the current use of smear microscopy or symptom screening alone. In addition, the MODS assay was able to accurately exclude active TB among patients who were smear-negative and who might

then be considered for isoniazid preventive therapy (IPT) if HIV-positive.(30) Scale-up of IPT in high HIV-prevalent settings has been hampered, in part, by concerns over accurate exclusion of active TB.(31,32) The MODS assay provides a simple, low-cost, rapid method for addressing this concern with a negative predictive value of 94-99% in HIV-positive TB suspects in our study.

Another important benefit of the MODS assay in this setting is the ability to rapidly diagnose and exclude MDR-TB. Numerous studies have shown that nearly half of all MDR-TB/HIV co-infected patients succumb to their disease within 30-60 days, before a diagnosis can be made using conventional methods.(33) Earlier diagnosis – in 9 days, as compared to 70 days – allows for patients to commence appropriate therapy sooner, thereby potentially saving lives. In addition, prompt identification of MDR-TB patients can facilitate implementation of infection control measures – such as isolation or discharge from hospital to community-based treatment programs of MDR-TB – allowing for reduced transmission of disease among highly-vulnerable patients, especially in high-HIV-prevalent settings.

Concerns about biosafety with MODS have been raised.(34) However, the MODS assay is performed directly on processed sputum in a sealed plastic bag that does not require further manipulation once the specimen has been inoculated.(35) Thus, unlike indirect DST methods which require secondary inoculation of cultured *M.tuberculosis* organisms, technicians need not handle specimens after plating. An added benefit to consider is the ability to isolate the organism directly from the MODS wells, if desired, for further species typing, genotypic testing, or second-line drug-susceptibility testing. The latter is particularly relevant in South Africa where rates of and mortality from extensively drug-resistant (XDR)-TB are high. With development of a "MODS kit" underway (D. Moore, personal communication), the MODS assay may offer an

option for decentralization of culture-based diagnosis of TB and MDR-TB to peripheral health centers in resource-constrained settings.

This study has limitations that must be considered. First, although sensitivity was high in smear-positive patients, overall sensitivity was lower than in other published studies. Reasons for this are unclear, but may be due to the number or type of sputum samples we collected from each patient (single spot sample), or sample storage, processing, and/or splitting, which may have significantly reduced the bacillary volume in each inoculum. Sensitivity may be improved slightly by performing multiple MODS assays, similar to other evaluations of rapid molecular tests on sputum.(36,37) Specificity has also been improved recently with revising of the MODS platform to include a microtitre well containing p-nnitrobenzoic acid (PNB), which specifically inhibits growth of *M.tuberculosis*. The absence of growth in PNB wells, combined with cord-formation in non-PNB wells, is specific for *M.tuberculosis*. Second, MODS results were not used for patient care in this study, so we were not able to evaluate the impact on TB or MDR-TB outcomes. Third, the daily observation of MODS plates, including weekends, may have contributed to faster turnaround time for MODS than in real-world settings. However, this approach allowed for greater comparability with the MGIT-960 automated system which is read continuously.

Lastly, the false-positive MODS results that occurred have important implications for both patient care and decentralization of the assay. These false-positive results likely represent cross-contamination from another positive specimen or from the H37Rv positive controls plated on each MODS plate. This underscores the importance of adequate staff training and a rigorous, well-supported quality assurance plan for all programs considering implementing MODS – or any other diagnostic method – in peripheral health centers.

In high HIV prevalence settings, the TB/HIV and MDR-TB epidemics are eroding gains achieved by antiretroviral therapy roll-out and are the major cause of mortality among co-infected patients.(1,38) Delays in diagnosis of TB and MDR-TB are the single largest barrier to improving outcomes for HIV-infected patients, with high, early mortality now well-documented for diverse settings.(13,39-41) The key to stemming the devastating dual epidemics of HIV and MDR-TB begins with improved case detection through earlier diagnosis, followed by initiation and support for appropriate treatment and implementation of infection control measures. The MODS assay can meet this critical need with relatively little infrastructure and training and, thus, should be evaluated for use in peripheral health centers where the majority of TB suspects are first seen and the impact is greatest.

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FIGURE LEGENDS

Figure 1a. Plot of time to *M.tuberculosis* growth for MODS and two reference standard culture methods (for samples that are positive on MODS and both reference standard cultures methods). Median turnaround time was significantly shorter for MODS than for the automated MGIT or Middlebrook agar cultures (9 days [IQR 6-12] vs. MGIT 16 days [IQR 12-48] vs. Middlebrook agar 29 days [IQR 20-41], respectively ($p<0.001$ for all pairwise comparisons). MGIT cultures are observed for 42 days; time-to-positivity beyond this time reflects contamination and repeat culture.

Figure 1b. Plot of time to MDR-TB diagnosis for MODS compared to reference standard 1% proportion method (For samples that are positive on MODS and at least one reference standard culture method).

Median turnaround time for MDR-TB diagnosis was significantly shorter for MODS than for the 1% proportion method (7 days [IQR 6–9] vs. 70 days [IQR 49–96)], $p<0.001$).

Table 1. Demographic and clinical characteristics of tuberculosis (TB) suspects

Characteristic	Total n (%)
Total N	**534**
Site of enrollment: Outpatient HIV and medical walk-in clinic	383 (72)
Inpatient ward	139 (26)
Other	12 (2)
Sex: Female	354 (66)
Male	180 (34)
Age: Median years (IQR)	38 (31–48)
Currently on TB treatment: Yes (i.e., treatment failure)	59 (11)
No	475 (89)
Contact with known TB case: Yes	119 (22)
No	411 (77)
Prior history of TB: Yes	131 (25)
No	397 (74)
HIV status*: Positive	388 (87)
Negative	58 (13)
Receiving antiretroviral therapy†: Yes	126 (33)
No	258 (66)
Duration on ART: median weeks (range)	19 (0–341)
CD4 cell count†: median cells/mm^3 (IQR)	161 (72–307)
≤50	64 (16)
51-200	140 (36)
201-350	68 (18)
>350	68 (18)

Unknown	48 (12)
TB symptoms present at screening[‡]:	
Cough	512 (96)
Fever	367 (69)
Night sweats	390 (73)
Weight loss	383 (72)
Chest pain	380 (71)
Culture-positive (i.e., confirmed TB case)	113 (21)
Sputum AFB smear result[§]:	
AFB smear-negative	50 (44)
AFB smear-positive	63 (56)
Scanty or 1+[‖]	19 (30)
2+ or 3+	44 (70)

IQR: Interquartile range

ART: antiretroviral therapy

AFB: acid-fast bacilli

* Among persons with known HIV status (n=466)

[†] At time of enrollment; among persons who are HIV-positive (n=388)

[‡] Persons could report more than 1 symptom, so total is greater than 100%

[§] Among persons with culture-confirmed TB (n=113)

[‖] Among persons with AFB smear-positive sputum result (n=63)

Table 2. Comparison of the microscopic-observation drug-susceptibility (MODS) assay with reference standard culture for detection of *M.tuberculosis*, by HIV status and sputum AFB smear status

MODS assay	**Combined reference standard** (agar or MGIT positive)	**Middlebrook agar**	**MGIT-960**
No. of samples positive for *M.tuberculosis* by reference standard method (%)	113 (21)	87 (16)	105 (20)
All patients (N=534)			
Sensitivity – % (95 CI)	85 (78–92)	94 (89–99)	85 (78–92)
Specificity – % (95 CI)	97 (95–99)	94 (92–96)	96 (94–98)
Positive predictive value – % (95 CI)	88 (82–94)	75 (67–83)	85 (78–92)
Negative predictive value – % (95 CI)	96 (94–98)	99 (98–100)	96 (94–98)
AFB smear-positive	**N=63**	**N=58**	**N=61**
Sensitivity – % (95 CI)	95 (90–100)	95 (89–100)	97 (92–100)
Specificity – % (95 CI)[*]	N/A	N/A	N/A
Positive predictive value – % (95 CI)	100 (94–100) [†]	100 (93–100) [†]	100 (94–100) [†]
Negative predictive value – % (95 CI)[*]	N/A	N/A	N/A
AFB smear-negative (n=470)			
Sensitivity – % (95 CI)	72 (60–84)	93 (84–100)	68 (54–82)
Specificity – % (95 CI)	97 (95–99)	95 (93-97)	96 (95-98)
Positive predictive value – % (95 CI)	74 (61–86)	55 (41-69)	67 (53-80)
Negative predictive value – % (95 CI)	97 (95–99)	99 (98-100)	97 (95-98)
HIV-positive (n=388) [‡]			
Sensitivity – % (95 CI)	88 (82–95)	96 (92–100)	89 (82–95)
Specificity – % (95 CI)	97 (95–99)	93 (90–96)	96 (93–98)

Positive predictive value – % (95 CI)	89 (83–96)	77 (68–85)	86 (79–93)
Negative predictive value – % (95 CI)	96 (94–98)	99 (98–100)	97 (94–99)
HIV-negative (n=58)			
Sensitivity – % (95 CI)	90 (71–100)	80 (55–100)	100 (69–100) [†]
Specificity – % (95 CI)	100 (93–100) [†]	100 (92–100) [†]	100 (93–100) [†]
Positive predictive value – % (95 CI)	100 (66–100) [†]	100 (63–100) [†]	100 (69–100) [†]
Negative predictive value – % (95 CI)	98 (94–100)	96 (90–100)	100 (93–100) [†]

AFB: acid-fast bacilli

MGIT: Mycobacterial Growth Indicator Tube

95 CI: 95% confidence interval

* Specificity and negative predictive value among smear-positive cases not calculated

[†] Two-sided 95% confidence interval (using exact method)

[‡] HIV status unknown for 87 subjects (8 TB cases, 79 non-TB cases)

Table 3. Drug-susceptibility results from the MODS Assay compared with the reference standard method, by sputum smear status

Measure	Isoniazid	Rifampin	Isoniazid + Rifampin (Multidrug-resistance)
No. of samples*	60	60	60
No. resistant (prevalence)	13 (21%)	14 (23%)	11 (18%)
Sensitivity – % (95 CI)	100 (66-100)†	100 (59-100)†	100 (59-100)†
Specificity – % (95 CI)	92 (85-99)	87 (78-96)	93 (85-99)
Positive predictive value – % (95 CI)	69 (44-94)	50 (24-76)	64 (35-92)
Negative predictive value – % (95 CI)	100 (92-100)†	100 (92-100)†	100 (93-100)†
Kappa value	78 (58-98)	61 (35-86)	74 (50-98)

95 CI: 95% confidence interval (97.5% confidence interval)

* Analysis limited to samples with positive MODS culture since isoniazid and rifampin wells are only observed if drug-free broth wells are positive for *M.tuberculosis*

† Two-sided 95% confidence interval (using exact method)

Appendix: Study Methods

Setting

Study subjects were recruited from Tugela Ferry, South Africa, a rural community within KwaZulu-Natal province where the incidence of TB is nearly 1100 per 100,000 population and more than 70% of TB cases are HIV co-infected. Reported MDR-TB incidence (inclusive of XDR-TB) was 74 cases per 100,000 population in 2008.(13)

Laboratory methods

M.tuberculosis detection

Upon receipt, sputum samples were digested and concentrated using the by the N-acetyl-L-cysteine (NALC)–sodium hydroxide (NaOH) method. Microscopic examination of the sediment was performed using the auramine fluorescent stain as well as the Ziehl-Neelson smear for the detection of acid-fast bacilli (AFB). The re-suspended sediment was then divided for parallel testing by MODS and two standard culture techniques: Middlebrook 7H10 agar plates and BACTEC™ MGIT™ 960 broth (Becton Dickinson, Sparks, MD, USA).

Middlebrook agar plates were sealed in CO_2-permeable bags and incubated in 5% CO_2 at 37°C and read at three weeks and six weeks for *M.tuberculosis* growth. Cultures that exhibited no growth by 6 weeks, or became contaminated were discarded.

MGIT broth tubes were incubated at 37°C in an automatic incubator and continuously monitored for 42 days for *M.tuberculosis* growth, per manufacturer recommendations. MGIT cultures that were contaminated prior to 42 days were re-

decontaminated and re-cultured. All positive cultures by MGIT were identified as *Mycobacterium tuberculosis* by using niacin and nitrate reductase tests.

The MODS assay was performed in accordance with published standard operating procedures,(28) with minor variations as noted. Broth cultures were prepared in 24-well tissue culture plates, each containing 900 μL Middlebrook 7H9 broth, OADC (oxalic acid, albumin, dextrose, and catalase) growth supplement, and PACT (polymyxin B, amphotericin B, carbecillin, trimethoprim) antibiotic supplement. For each patient sample, 4 wells were used: 2 drug-free wells, 1 with isoniazid at 0.4 μg/ml, and 1 with rifampicin at 1 μg/ml. MODS cultures were examined using an inverted light microscope at 40x magnification every day from day 4 through day 21. To minimize cross-contamination and occupational exposure, MODS plates were sealed in CO2 permeable bags after inoculation and were examined through the bag. Positive MODS cultures were identified by presence of characteristic cord formation in the drug-free control wells. A subset of samples underwent only MODS culture testing (i.e., drug-free wells) without isoniazid and rifampin testing. MODS culture performance was analyzed for all samples.

For study purposes, positive MODS wells were plated-out onto Middlebrook 7H10 plates and examined for *M.tuberculosis* growth. Standard biochemical tests (niacin and nitrate reductase) were employed to confirm *M.tuberculosis* species. In addition, if contamination was suspected, MODS wells were plated-out on blood and chocolate agar plates.

All MODS procedures were performed by experienced laboratory technicians who received further training in the MODS assay. All study staff were blinded to results of standard culture and DST methods, which were performed in parallel by the routine

TB laboratory (TB laboratory staff were also blinded to MODS results). Results are reported for specimens tested after staff achieved proficiency with the MODS assay.

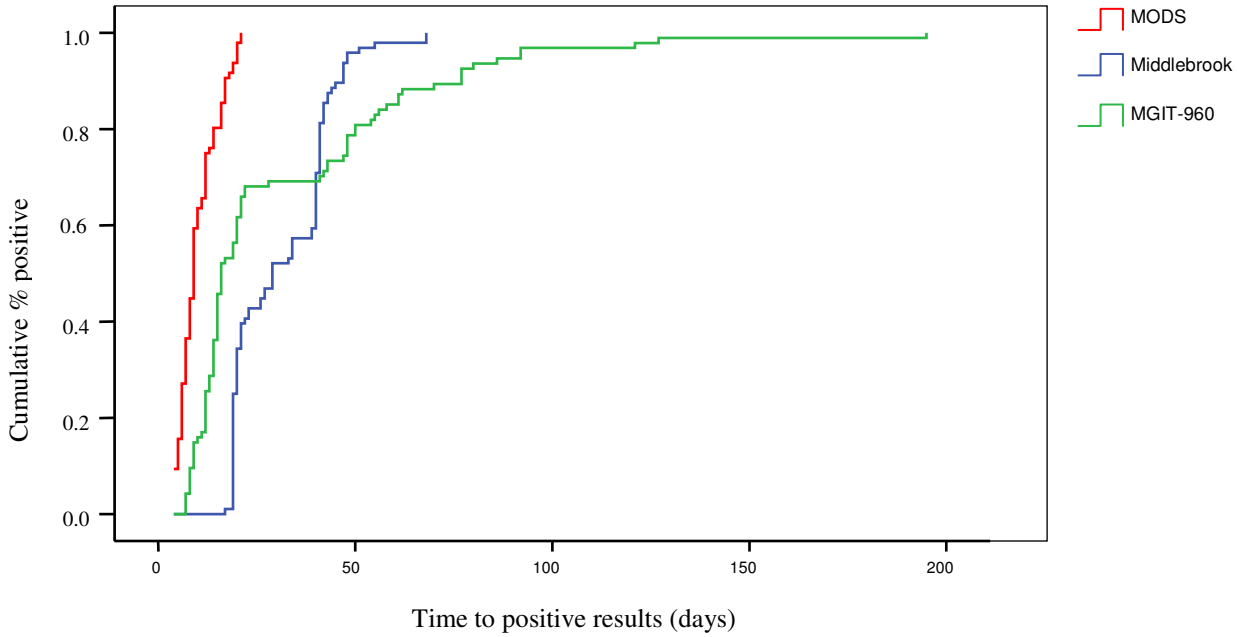
MODS
Middlebrook
MGIT-960
Cumulative % positive
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
Time to positive results (days)

Figure 1a.

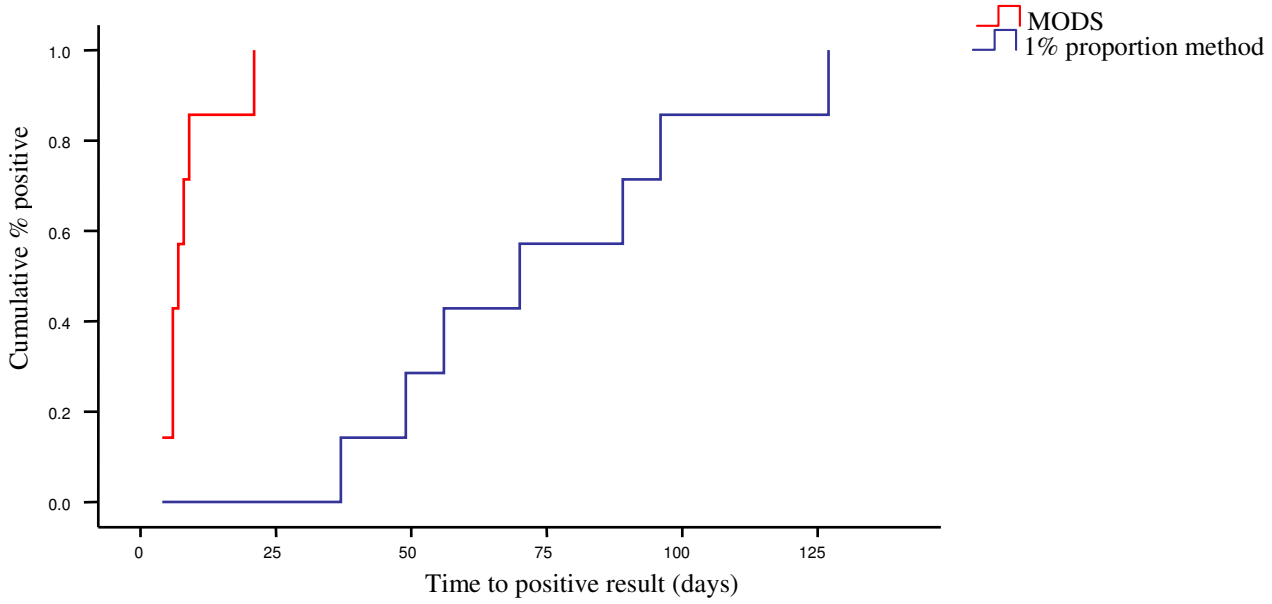
MODS
1% proportion method
Cumulative % positive
1.0
0.8
0.6
0.4
0.2
0.0
0
25
50
75
100
125
Time to positive result (days)

Figure 1b.