ORIGINAL ARTICLE

Microscopic-Observation Drug-Susceptibility Assay for the Diagnosis of TB

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ABSTRACT

BACKGROUND

New diagnostic tools are urgently needed to interrupt the transmission of tuberculosis and multidrug-resistant tuberculosis. Rapid, sensitive detection of tuberculosis and multidrug-resistant tuberculosis in sputum has been demonstrated in proof-of-principle studies of the microscopic-observation drug-susceptibility (MODS) assay, in which broth cultures are examined microscopically to detect characteristic growth.

METHODS

In an operational setting in Peru, we investigated the performance of the MODS assay for culture and drug-susceptibility testing in three target groups: unselected patients with suspected tuberculosis, prescreened patients at high risk for tuberculosis or multidrug-resistant tuberculosis, and unselected hospitalized patients infected with the human immunodeficiency virus. We compared the MODS assay head-to-head with two reference methods: automated mycobacterial culture and culture on Löwenstein–Jensen medium with the proportion method.

RESULTS

Of 3760 sputum samples, 401 (10.7%) yielded cultures positive for *Mycobacterium tuberculosis*. Sensitivity of detection was 97.8% for MODS culture, 89.0% for automated mycobacterial culture, and 84.0% for Löwenstein–Jensen culture (P<0.001); the median time to culture positivity was 7 days, 13 days, and 26 days, respectively (P<0.001), and the median time to the results of susceptibility tests was 7 days, 22 days, and 68 days, respectively. The incremental benefit of a second MODS culture was minimal, particularly in patients at high risk for tuberculosis or multidrug-resistant tuberculosis. Agreement between MODS and the reference standard for susceptibility was 100% for rifampin, 97% for isoniazid, 99% for rifampin and isoniazid (combined results for multidrug resistance), 95% for ethambutol, and 92% for streptomycin (kappa values, 1.0, 0.89, 0.93, 0.71, and 0.72, respectively).

CONCLUSIONS

A single MODS culture of a sputum sample offers more rapid and sensitive detection of tuberculosis and multidrug-resistant tuberculosis than the existing gold-standard methods used.

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Every year, 1.7 million people die of tuberculosis, a curable disease.[1] The poor are disproportionately affected, and tuberculosis further impoverishes individual people and societies. Goal 6 of the Millennium Development Goals of the United Nations includes the halting and reversal of the rising incidence of tuberculosis, and the Stop TB Partnership aims to halve the prevalence of tuberculosis and resulting deaths by 2015.[2] Existing control strategies miss important opportunities to interrupt transmission. Improved tuberculosis detection and early identification of multidrug-resistant tuberculosis are key gaps.

Sputum-smear–based diagnosis under the Direct Observation of Therapy (Short Course) (DOTS) strategy of the World Health Organization for global tuberculosis control misses half of incident cases at first presentation. Transmission continues until cases are detected with more advanced (smear-positive) disease and are correctly treated. Multidrug-resistant tuberculosis increases morbidity and mortality and, through treatment failure,[3] facilitates continuing transmission from patients who (like their health care providers) wrongly believe they are being cured. The use of treatment failure to prompt drug-susceptibility testing relies on the same illness-threshold effect as waiting for smears to become positive in patients with negative smears.[4] Both scenarios could be addressed by appropriate new diagnostics.[5]

The microscopic-observation drug-susceptibility (MODS) assay for the detection of tuberculosis and multidrug-resistant tuberculosis, directly from sputum, relies on three principles: first, that *Mycobacterium tuberculosis* grows faster in liquid medium than in solid medium; second, that characteristic cord formation can be visualized microscopically in liquid medium at an early stage; and third, that the incorporation of drugs permits rapid and direct drug-susceptibility testing concomitantly with the detection of bacterial growth.

In proof-of-principle studies, the MODS assay distinguished patients with tuberculosis from healthy controls.[6,7] In accordance with recommendations,[8] we undertook an operational evaluation of the MODS assay in order to answer two questions: How well does it distinguish between patients with and those without active tuberculosis among those with suspected tuberculosis? Among patients with active tuberculosis, how well does it distinguish drug-resistant disease from drug-sensitive disease?

Given the challenges of the evaluation of techniques for the diagnosis of tuberculosis,[9-13] our study was conducted in accordance with the emerging consensus about the design and reporting of diagnostic-test evaluations[14-18] and the minimum standards required for diagnostic trials of tuberculosis.[8,19,20]

METHODS

STUDY PATIENTS AND SETTING

The study was conducted in Lima, Peru, from April 2003 through July 2004 in three target groups, with consecutive recruitment. The first group consisted of otherwise unselected patients who presented with suspected tuberculosis to the National TB Programme at 10 government clinics in north Lima. The second consisted of patients who presented with suspected tuberculosis to the National TB Programme at five government clinics in east Lima and who were at high risk for tuberculosis or multidrug-resistant tuberculosis. Inclusion in this group required the presence of one or more constitutional symptoms (fever, weight loss, night sweats, hemoptysis) or one risk factor for tuberculosis or multidrug-resistant tuberculosis (prior treatment for tuberculosis, known contact with a patient with tuberculosis, infection with human immunodeficiency virus [HIV], employment as a health care or prison worker, hospitalization during the previous year, or any previous incarceration). The third group consisted of otherwise unselected hospitalized patients with HIV infection at two Lima hospitals, regardless of the diagnosis on admission. Exclusion criteria for all groups were an age under 18 years or an inability or unwillingness to give written informed consent. Study protocol and consent forms were approved by the institutional review boards of Universidad Peruana Cayetano Heredia, Asociación Benéfica PRISMA, Dirección de Salud–III Lima Norte and Dirección de Salud–IV Lima Este (regional Ministry of Health), Hospital Nacional Hipolito Unanue, Hospital Nacional General Arzobispo Loayza, Johns Hopkins Bloomberg School of Public Health, and Imperial College London.

SAMPLE COLLECTION

Patients with suspected tuberculosis and prescreened patients at high risk for tuberculosis or

multidrug-resistant tuberculosis submitted two samples of sputum to the National TB Programme for routine Ziehl–Neelsen staining and consented to their subsequent use in the study (at Universidad Peruana Cayetano Heredia). Among the hospitalized patients with HIV infection, two samples per patient were collected exclusively for the study, of which 20 were from gastric washing in 15 patients who were unable to provide adequate sputum samples.

Patients with suspected tuberculosis and prescreened patients at high risk for tuberculosis or multidrug-resistant tuberculosis who were eligible for the study were not recruited if they presented at the clinic outside of normal working hours (>90% of excluded patients), declined to participate (<5%), or if the clinic staff were unavailable (<5%). Numbers of eligible patients were derived from data extracted from National TB Programme log entries during the recruitment period; the numbers may have been overestimated, since follow-up patients (who were ineligible) were not always clearly identified. Of the 25 hospitalized patients with HIV infection who were eligible but not recruited, more than 90% were incapable of providing informed consent, owing to their clinical condition, or were receiving ongoing treatment for tuberculosis. Most, but not all, patients submitted two sputum samples.

LABORATORY METHODS

Detection of M. tuberculosis

Sputum samples were decontaminated according to the sodium hydroxide–*N*-acetyl-L-cysteine method.[21] An aliquot was used for microscopical examination of auramine-stained sputum smears, and the remainder was used for parallel Löwenstein–Jensen culture, automated mycobacterial culture, and MODS culture (see Fig. I in the Supplementary Appendix, available with the full text of this article at www.nejm.org). Löwenstein–Jensen culture and automated mycobacterial culture with the use of the MBBacT system (bioMérieux) were selected because they are reference methods commonly used in developing and industrialized countries, respectively. After inoculation of 250 μl of decontaminant, Löwenstein–Jensen slants were incubated at 37°C and examined twice weekly from day 7 through day 60.[21] MBBacT bottles were inoculated with 500 μl of decontaminant, and cultures were monitored continuously for 42 days according to the recommendations of the manufacturer.

The MODS assay was performed as described previously.[6,7] Briefly, broth cultures were prepared in 24-well tissue-culture plates (Becton Dickinson), each containing 720 μl of decontaminant, Middlebrook 7H9 broth (Becton Dickinson), oxalic acid, albumin, dextrose, and catalase (OADC) (Becton Dickinson), and polymyxin, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA) (Becton Dickinson). For each sample, 12 wells were used: in 4 control wells, no drug was added, and each of the remaining 8 wells contained one of four drugs at one of two concentrations tested. The cultures were examined under an inverted light microscope at a magnification of 40× every day (except Saturday and Sunday) from day 4 to day 15, on alternate days from day 16 to day 25, and twice weekly from day 26 to day 40. To minimize cross-contamination and occupational exposure, plates were permanently sealed inside plastic ziplock bags after inoculation and were subsequently examined within the bag. Positive cultures were identified by cord formation, characteristic of *M. tuberculosis* growth, in liquid medium in drug-free control wells, as described previously.[6,7,22] Nontuberculous mycobacteria were recognized by their lack of cording or, for *M. chelonae* (which is the only nontuberculous mycobacteria that does form cords), by rapid overgrowth by day 5. Fungal or bacterial contamination was recognized by rapid overgrowth and clouding in wells.

If contamination was detected, the original sample was cultured again after being decontaminated once more. Spacer oligonucleotide typing (spoligotyping), polymerase chain reaction with multiple primers,[23] or both were applied to all isolates from each of the three types of cultures in order to confirm the presence of *M. tuberculosis*.

Drug-Susceptibility Testing

Direct drug-susceptibility testing was performed with the use of the MODS assay, as previously described.[6,7] Growth in drug-free control wells but not in drug-containing wells indicated susceptibility. The drug concentrations used were as follows: isoniazid, 0.1 and 0.4 μg per milliliter; rifampin, 1 and 2 μg per milliliter; ethambutol, 2.5 and 5.0 μg per milliliter; and streptomycin, 2 and 6 μg per milliliter. Drug-sensitive control strains were tested daily. Indirect drug-susceptibility testing was performed with the use of the proportion method[21] for isolates from Löwenstein–Jensen culture (by an external laboratory)

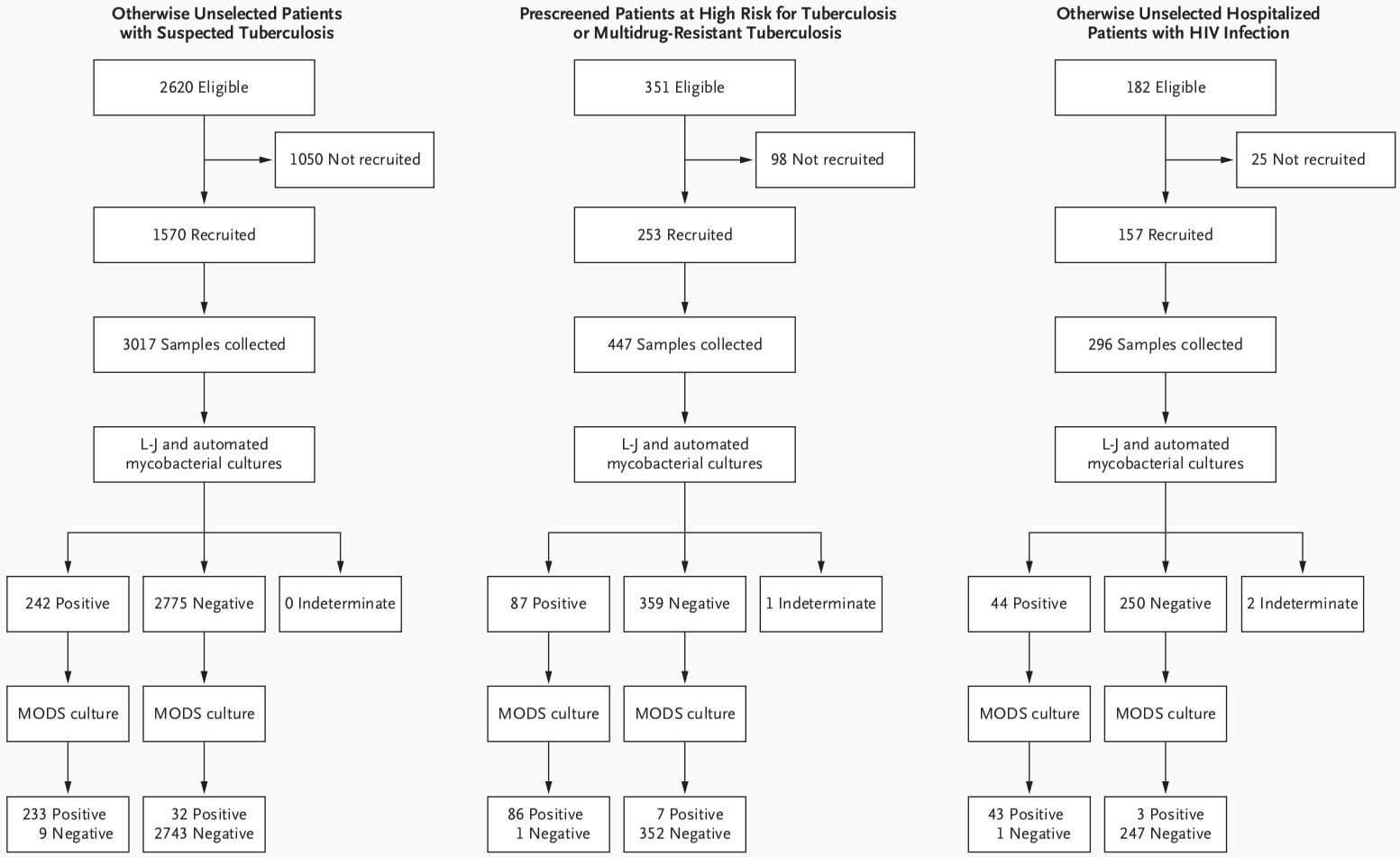

Figure 1. Recruitment of Patients and Culture Results.

For Löwenstein–Jensen (L-J) and automated mycobacterial cultures, positive cultures were those that were positive according to either method or both; negative cultures were those that were negative according to both methods or negative according to one method and indeterminate according to the other; and indeterminate cultures were those that were indeterminate according to both methods. For patients with suspected tuberculosis, the 242 reference-standard culture-positive samples included 4 false positive samples arising from cross-contamination[36] (1 in Löwenstein–Jensen culture alone, 1 in automated mycobacterial culture alone, and 2 in both automated mycobacterial culture and MODS culture), and the 32 culture-positive samples in MODS culture alone included 7 that were false positive owing to cross-contamination. For the prescreened patients at high risk for tuberculosis or multidrug-resistant tuberculosis, the 87 reference-standard culture-positive samples included 1 false positive sample in both automated mycobacterial culture and MODS culture, and the 7 samples that were culture-positive in MODS culture alone included 2 false positive cultures. The indeterminate cultures for the prescreened patients and the hospitalized patients with HIV infection were designated as such because cultures from all three methods were repeatedly contaminated by bacterial overgrowth (two with rapid-growing nontuberculous mycobacteria).

and with the automated MBBacT system[24-29] for isolates from the automated mycobacterial culture. For purposes of discrepant analysis, the microplate alamar blue assay[30-35] was performed in parallel, both for all Löwenstein–Jensen isolates and for isolates from drug-free control wells in the MODS assay. All procedures were performed by six staff members of the mycobacteriology laboratory who were unaware of the results of the other tests.

STATISTICAL ANALYSIS

Data were analyzed with the use of Stata 7 software, with the sample as the unit of analysis, to reflect the operational performance of a routine service laboratory. The Wilcoxon signed-rank test was used to compare the times to each end point among the three methods. A P value of less than 0.05 was used to indicate statistical significance. The concordance of susceptibility results was determined with the use of the sensitivity, specificity, and positive and negative predictive values for the detection of resistance (with 95% confidence intervals [CIs]), as well as with kappa values.

To address the inherent difficulty of evaluating a test that is more sensitive than the reference tests, and to minimize incorporation bias (the use of results from the investigational test as part of the reference result), we previously undertook a comprehensive microbiologic, mo-

Table 1. Demographic Characteristics, Prevalence of Disease, and Sensitivity, Specificity, and Predictive Values for the Detection of *Mycobacterium tuberculosis* in Sputum.*

Characteristic	All Patients (N=1980)	Patients with Suspected Tuberculosis (N=1570)	Prescreened Patients at High Risk for Tuberculosis or Multidrug-Resistant Tuberculosis (N=253)	Hospitalized Patients with HIV Infection (N=157)
Median age — yr	32	32	34	32
Female sex — %	58.1	60.2	56.6	38.5
Positive smear for acid-fast bacilli — no./no. of samples (%)	220/3757 (5.9)	142/3017 (4.7)	55/446 (12.3)	23/294 (7.8)
Culture positive for *M. tuberculosis* — no./no. of samples (%)	401/3757 (10.7)	263/3017 (8.7)	91/446 (20.4)	47/294 (16.0)
MODS assay — % (95% CI)				
Sensitivity	97.8 (97.3–98.2)	97.3 (96.8–97.9)	98.9 (97.9–99.9)	97.9 (96.2–99.5)
Specificity	99.6 (99.5–99.8)	99.7 (99.5–99.9)	99.2 (98.3–100)	100 (100–100)
PPV	97.0 (96.5–97.6)	96.6 (96.0–97.3)	96.8 (95.1–98.4)	100 (100–100)
NPV	99.7 (99.6–99.9)	99.8 (99.6–99.9)	99.7 (99.2–100)	99.6 (98.9–100)
Automated mycobacterial culture — % (95% CI)				
Sensitivity	89.0 (88.0–90.0)	87.8 (86.7–89.0)	92.3 (89.8–94.8)	89.4 (85.8–92.9)
Specificity	99.9 (99.8–100)	99.9 (99.8–100)	99.7 (99.2–100)	100 (100–100)
PPV	98.9 (98.6–99.2)	98.7 (98.3–99.1)	98.8 (97.8–99.8)	100 (100–100)
NPV	98.7 (98.3–99.1)	98.9 (98.5–99.2)	98.1 (96.8–99.3)	98.0 (96.4–99.6)
Löwenstein–Jensen culture — % (95% CI)				
Sensitivity	84.0 (82.9–85.2)	82.5 (81.2–83.9)	89.0 (86.1–91.9)	83.0 (78.7–87.3)
Specificity	100 (99.9–100)	100 (99.9–100)	100 (100–100)	100 (100–100)
PPV	99.7 (99.5–99.9)	99.5 (99.3–99.8)	100 (100–100)	100 (100–100)
NPV	98.1 (97.7–98.6)	98.4 (97.9–98.8)	97.3 (95.8–98.8)	96.9 (94.9–98.9)

* For sensitivity and specificity of detection and predictive-value calculations, a positive reference result was defined as a positive culture according to at least one method for which cross-contamination had been conclusively ruled out; a negative reference result was defined as any sample in which all three culture methods yielded negative results or two were negative and the third indeterminate, owing to repeated bacterial or fungal overgrowth, or a sample for which cross-contamination was demonstrated to be the only positive cause of a positive culture.[36] Upper 95% CIs exceeding 100% were rounded to 100%. PPV denotes positive predictive value, and NPV negative predictive value.

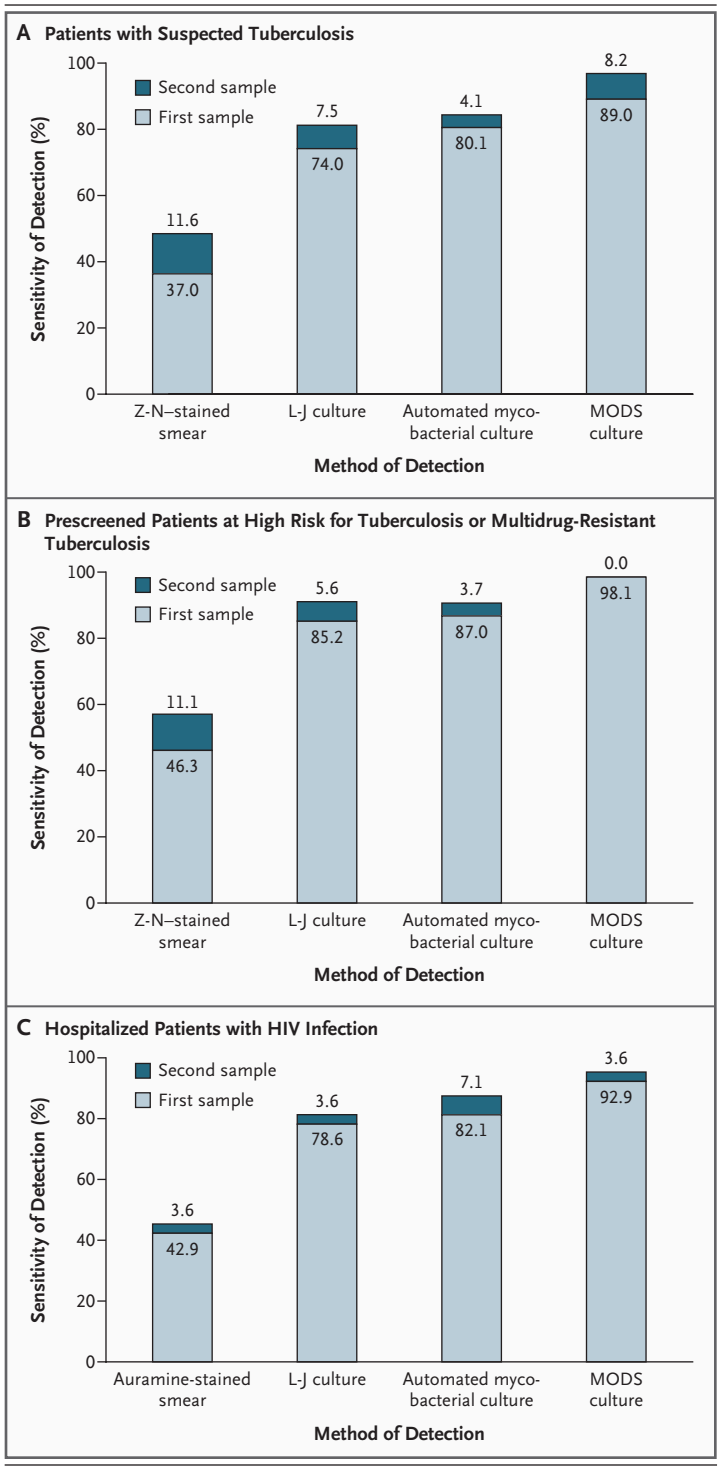

Figure 2. Incremental Benefit of One and Two Sputum Cultures as Compared with Sputum-Smear Microscopy.

The percentage of cases detected during examination of the first smear or culture is shown, as well as the additional (not total) percentage detected during examination of a second smear or culture. For patients with suspected tuberculosis (Panel A) and prescreened patients at high risk for tuberculosis or multidrug-resistant tuberculosis (Panel B), examination of smears stained with the Ziehl–Neelsen (Z-N) stain was performed at the local laboratory of the National TB Programme before the sample was retrieved for study purposes. For samples from the hospitalized patients with HIV infection (Panel C), microscopical examination of sputum smears stained with auramine but not Z-N stain was performed at Universidad Peruana Cayetano Heredia, because these patients were not recruited through the National TB Programme. L-J denotes Löwenstein–Jensen.

lecular, and epidemiologic investigation of the discordant cultures.[36] We identified 17 cross-contaminated cultures (12 MODS cultures, 4 automated mycobacterial cultures, and 1 Löwenstein–Jensen culture) from 14 samples. For sensitivity and specificity of detection and predictive-value calculations for each of the three methods, a positive reference result was defined as a positive culture according to at least one method for which cross-contamination had been conclusively ruled out.[36] A negative reference result was defined as any sample in which all three culture methods yielded negative results or in which two were negative and the third indeterminate, owing to repeated bacterial or fungal overgrowth, or a sample for which cross-contamination was demonstrated to be the only cause of the positive culture.[36]

Thus, the 17 false positive cultures were defined as positive in calculations of performance characteristics for the relevant methods, but the reference result for the 14 samples was determined to be negative. McNemar's χ^2 test was used to compare the sensitivities of detection of the three methods.

DEFINITION OF REFERENCE SUSCEPTIBILITY TEST RESULTS

Concordant results from automated mycobacterial culture and the proportion method were regarded as the reference result for drug susceptibility. Discordant results from automated mycobacterial culture and the proportion method were resolved by means of discrepant analysis, with the use of the two parallel results from the microplate alamar blue assay. If both results from this assay agreed, that result was used as the reference result; if not, the strain was designated indeterminate (see Table I in the Supplementary Appendix).

RESULTS

PATIENTS AND SAMPLES

Recruitment of patients and culture results are shown in Figure 1. Demographic characteristics and tuberculosis diagnoses are shown in Table 1 according to study group.

SENSITIVITY AND SPECIFICITY OF DETECTION

Of the 3760 sputum samples collected, 401 (10.7%) were positive for *M. tuberculosis* cultures, 3356 were negative, and 3 were indeterminate (and were removed from analysis), because all three types of cultures were repeatedly contaminated by bacterial overgrowth. MODS culture had a greater overall sensitivity of detection than either automated mycobacterial culture or Löwenstein–Jensen culture (97.8%, 89.0%, and 84.0%, respectively; P<0.001); this difference was maintained in all groups. The overall specificity of detection was 99.6% for MODS culture, 99.9% for automated mycobacterial culture, and 100.0% for Löwenstein–Jensen culture. Predictive values and data according to group are shown in Table 1, and in Figure II of the Supplementary Appendix.

ADDED VALUE OF SECOND CULTURE FOR SENSITIVITY OF DETECTION

The incremental benefit of a second smear for acid-fast bacilli and a second sputum culture is shown in Figure 2. A second MODS culture detected an additional 8.2% of cases among patients with suspected tuberculosis but offered no added value among prescreened patients at high risk for tuberculosis or multidrug-resistant tuberculosis.

TIME TO CULTURE POSITIVITY

Of the 401 sputum samples positive for *M. tuberculosis*, 325 were culture-positive according to all three methods and were thus included in the head-to-head analysis of time to culture positivity (Fig. 3). The median time to culture positivity was significantly shorter for MODS than for the automated mycobacterial or Löwenstein–Jensen cultures (7 days [interquartile range, 6 to 8] vs. 13 days [interquartile range, 10 to 16] and 26 days [interquartile range, 21 to 33], respectively; P<0.001). Smear status had a clinically unimportant, though significant, effect on time to culture positivity in MODS culture (median, 6 days for a smear-positive sample vs. 7 days for a smear-

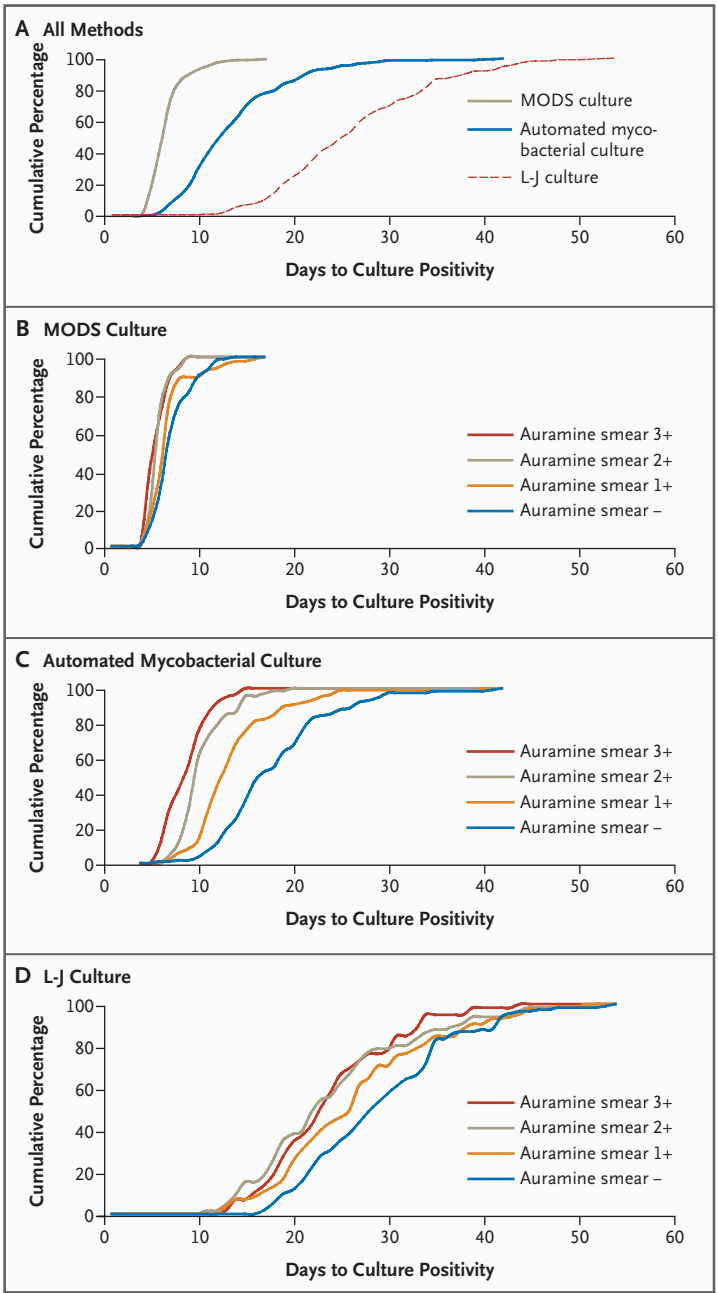

Figure 3. Cumulative Percentages of the Time to Culture Positivity for 325 Culture-Positive Samples According to Culture Method (Panel A) and the Effect of the Quantitative Status of Sputum Smears for Acid-Fast Bacilli (Panels B, C, and D).

The percentages of cultures that were positive at days 7, 14, and 21 were 74%, 99%, and 100%, respectively, in MODS culture; 7%, 62%, and 89% in automated mycobacterial culture; and 0%, 5%, and 28% in Löwenstein–Jensen (L-J) culture (Panel A). In Panels B, C, and D, "auramine smear –" was defined by the presence of fewer than 10 acid-fast bacilli per 100 fields, "1+" 10 to 99 acid-fast bacilli per 100 fields, "2+" 1 to 10 acid-fast bacilli per field, and "3+" more than 10 acid-fast bacilli per field. One field was equivalent to the examination of one carbol-fuchsin–stained smear at a magnification of 1000×.

Table 2. Susceptibility Results from the MODS Assay and Concordance with the Gold-Standard Methods.*

Measure	All Patients	Patients with Suspected Tuberculosis	Prescreened Patients at High Risk for Tuberculosis or Multidrug-Resistant Tuberculosis	Hospitalized Patients with HIV Infection
Rifampin, 1 μg/ml				
No. of samples	338			
Resistant samples — % (95% CI)	10.7 (7.4–13.9)	11.0 (6.8–15.1)	13.8 (6.2–21.3)	2.6 (0–7.5)
Kappa value	1.0	1.0	1.0	1.0
Sensitivity — % (95% CI)	100 (100–100)	100 (100–100)	100 (100–100)	100 (100–100)
Specificity — % (95% CI)	100 (100–100)	100 (100–100)	100 (100–100)	100 (100–100)
PPV — % (95% CI)	100 (100–100)	100 (100–100)	100 (100–100)	100 (100–100)
NPV — % (95% CI)	100 (100–100)	100 (100–100)	100 (100–100)	100 (100–100)
Isoniazid, 0.4 μg/ml				
No. of samples	334			
Resistant samples — % (95% CI)	19.5 (15.2–23.7)	14.4 (9.7–19.0)	30.4 (20.2–40.5)	25.6 (11.9–39.4)
Kappa value	0.89	0.92	0.84	0.86
Sensitivity — % (95% CI)	84.6 (80.8–88.5)	90.3 (86.4–94.3)	79.2 (70.2–88.1)	80.0 (67.5–92.6)
Specificity — % (95% CI)	99.6 (99.0–100)	99.5 (98.5–100)	100 (100–100)	100 (100–100)
PPV — % (95% CI)	98.2 (96.8–99.6)	96.6 (94.1–99.0)	100 (100–100)	100 (100–100)
NPV — % (95% CI)	96.4 (94.4–98.4)	98.4 (96.7–100)	91.7 (85.6–97.8)	91.7 (85.6–97.8)
Rifampin and isoniazid†				
No. of samples	334			
Resistant samples — % (95% CI)	10.4 (7.1–13.6)	10.5 (6.4–14.6)	13.8 (6.2–21.3)	2.6 (0–7.5)
Kappa value	0.93	0.95	0.89	1.0
Sensitivity — % (95% CI)	88.6 (85.2–92.0)	91.3 (87.6–95.0)	81.8 (73.3–90.3)	100 (100–100)
Specificity — %	100 (100–100)	100 (100–100)	100 (100–100)	100 (100–100)
PPV — %	100 (100–100)	100 (100–100)	100 (100–100)	100 (100–100)
NPV — % (95% CI)	98.7 (97.5–99.9)	99.0 (97.6–100)	97.1 (93.5–100)	100 (100–100)

negative sample; P<0.001). Though samples that were culture-negative (69) or contaminated (7) according to at least one method were excluded from this analysis, results were unchanged by their inclusion (data not shown).

BACTERIAL AND FUNGAL CONTAMINATION OF CULTURES

The median time from sample collection to sample processing was 3 days, and most but not all samples were refrigerated en route. At least one culture per sample was contaminated in 739 of 3760 samples (20.0%), though in only 63 samples (1.7%) were all cultures by the three methods contaminated. The proportion of initially contaminated samples was 8.1% (95% CI, 7.2 to 9.0) for MODS culture, 4.4% (95% CI, 3.8 to 5.3) for automated mycobacterial culture, and 14.2% (95% CI, 13.1 to 15.3) for Löwenstein–Jensen culture. However, ultimately contaminated (indeterminate) cultures were less frequent in MODS culture (6 cultures, 0.2%) than in either automated mycobacterial culture (11 cultures, 0.3%; P=0.01) or Löwenstein–Jensen culture (55 cultures, 1.5%; P<0.001). The median time from initial processing of samples to the results of culture testing for initially contaminated cultures was shorter for MODS culture (24 days; 95% CI, 19 to 28) than for automated mycobacterial culture (32 days; 95% CI, 25 to 39; P=0.03) or Löwenstein–Jensen culture (50 days; 95% CI, 44 to 56; P<0.001). Positive MODS cultures that were contaminated but able to be evaluated accounted for less than 2.5% of all positive cultures for the assay.

Table 2. (Continued.)

Measure	All Patients	Patients with Suspected Tuberculosis	Prescreened Patients at High Risk for Tuberculosis or Multidrug-Resistant Tuberculosis	Hospitalized Patients with HIV Infection
Ethambutol, 2.5 μg/ml				
No. of samples	327			
Resistant samples — % (95% CI)	10.1 (6.8–13.4)	10.8 (6.6–14.9)	6.6 (1.0–12.2)	13.5 (2.5–24.5)
Kappa value	0.71	0.78	0.71	0.3
Sensitivity — % (95% CI)	63.6 (58.4–68.9)	69.6 (63.4–75.7)	80.0 (71.0–89.0)	20.0 (7.1–32.9)
Specificity — % (95% CI)	99.0 (97.9–100)	99.5 (98.5–100)	97.2 (93.5–100)	100 (100–100)
PPV — % (95% CI)	87.5 (83.9–91.1)	94.1 (91.0–97.3)	66.7 (56.1–77.3)	100 (100–100)
NPV — % (95% CI)	96.0 (93.9–98.2)	96.5 (94.0–98.9)	98.6 (95.9–100)	88.9 (78.8–99.0)
Streptomycin, 2 μg/ml				
No. of samples	327			
Resistant samples — % (95% CI)	21.4 (17.0–25.9)	19.3 (14.0–24.5)	24.1 (14.6–33.5)	28.6 (13.6–43.5)
Kappa value	0.72	0.72	0.70	0.77
Sensitivity — % (95% CI)	65.7 (60.6–70.9)	63.4 (57.0–69.9)	68.4 (58.2–78.7)	70 (54.8–85.2)
Specificity — % (95% CI)	98.8 (97.7–100)	99.4 (98.4–100)	96.7 (92.7–100)	100 (100–100)
PPV — % (95% CI)	93.9 (91.3–96.5)	96.3 (93.8–98.8)	86.7 (79.2–94.2)	100 (100–100)
NPV — % (95% CI)	91.4 (88.3–94.4)	91.9 (88.3–95.6)	90.6 (84.2–97.1)	89.3 (79.0–99.5)

* For susceptibility testing by the MODS assay, 349 samples were available; the number included in the analysis for each drug varied owing to differences in the rates of culture positivity and to occasional contamination. Two concentrations were examined for each drug; of susceptibility-test cultures in each case, one concentration was clearly superior, with no useful information gained from the second concentration (data not shown). The percentage of samples that were resistant was determined by gold-standard reference tests (see Table 1 in the Supplementary Appendix). The kappa statistic describes the strength of agreement beyond that due to chance between data from the MODS assay and the reference results, as follows: 0.00 to 0.20, slight; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, substantial; and 0.81 to 1.00, high. Sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs) are for the detection of drug resistance. Upper 95% CIs exceeding 100% were rounded to 100%.

† Results for rifampin and isoniazid were combined in analyses for multidrug resistance.

DIRECT DRUG-SUSCEPTIBILITY TESTING

Valid drug-susceptibility testing in the MODS assay depends on the observed growth of *M. tuberculosis* in all four control wells, as was the case for 349 of 392 positive MODS cultures (89.0%). Of the 43 samples for which drug-susceptibility testing in the MODS assay was not possible, 28 (65.1%) were culture-negative according to automated mycobacterial culture and Löwenstein–Jensen culture. Resistance to rifampin was detected in 10.7% of all samples; to isoniazid, in 19.5%; to rifampin and isoniazid (combined to test for multidrug resistance), in 10.4%; to ethambutol, in 10.1%; and to streptomycin, in 21.4% (Table 2). The proportion of samples for which susceptibility results agreed between MODS culture and the gold-standard methods was 100% for rifampin, 96.7% for isoniazid, 98.8% for rifampin and isoniazid, 95.4% for ethambutol, and 91.7% for streptomycin. Overall median times from initial sample processing to the results of drug-susceptibility testing were 7 days for MODS culture, 22 days for automated mycobacterial culture, and 68 days for Löwenstein–Jensen culture.

DISCUSSION

This operational study extends and provides support for the findings of earlier proof-of-principle studies[6,7,22,37] and demonstrates that the MODS assay outperforms the gold-standard reference methods of developing and industrialized countries. For all three study groups, the MODS assay detected *M. tuberculosis* in sputum with greater sensitivity and speed and reliably identified multidrug-resistant tuberculosis strains in less time than did Löwenstein–Jensen or automated mycobacterial cultures. These data indicate that the

MODS assay could be considered for use in appropriate settings.

Our study was designed to address conventional pitfalls.[8,15,18,20,38] Specifically, it was performed in an appropriately broad group of patients with or without disease and in pertinent patient groups (without selection bias); all tests were performed in all patients (preventing verification bias); and all results were interpreted by staff members who were unaware of the other test results, using appropriate gold-standard reference methods for comparison.

The robustness of our study derives from its operational, real-world design. Meticulous resolution of discordant results is essential when an investigational diagnostic method is more sensitive than existing reference standards. Use of two established reference methods for comparison and two samples per patient facilitated the rigorous definition of true positive results, addressing the problem of incorporation bias. The high specificity and infrequent cross-contamination in the MODS assay[36] relate to the containment of the plates in ziplock bags and the absence of manipulation after inoculation, which also improve biologic security.

The greater sensitivity and speed of detection in MODS culture than in the gold standards were predicted on the basis of previous studies.[6,7,37] The increased sensitivity of liquid medium has long been known, and a discerning human eye can scrutinize cultures better than can automated systems with their use of necessarily rigid cutoff values. It is simpler to recognize the characteristic cord formation than to read a malarial smear; within 1 week, students training in our laboratory can comfortably and accurately read one well per minute, considerably faster than the time it takes to read a smear for acid-fast bacilli. Training in the MODS assay can be completed in less than 2 weeks (similar to training in Löwenstein–Jensen and automated mycobacterial cultures; training in drug-susceptibility testing with the proportion method takes several months). Beyond standard laboratory equipment, automated mycobacterial culture requires computer-linked automated culture incubators, whereas MODS culture requires only an inverted light microscope. As purchased by us, the cost of $2 per sample for MODS culture compares favorably with the $6 cost per sample for Löwenstein–Jensen culture and the proportion method and the cost of $52 per sample for automated mycobacterial culture; however, labor costs may be higher for MODS culture.

Increased sensitivity carries the risk of increased bacterial overgrowth (for MODS culture and automated mycobacterial culture), though even after repeated decontamination, the sensitivity and specificity of MODS culture were unaffected. High speed, sensitivity, and specificity, and the requirement for only one culture, all enhance tuberculosis rule-out procedures and potentially simplify tuberculosis-screening algorithms for use in patients with HIV infection before the initiation of prophylactic treatment with isoniazid. If a MODS culture is negative on day 15, there is a 99.7% chance that the sample is truly culture-negative. Thus, we believe that a negative MODS culture can be discarded after 3 weeks.

In settings with a high tuberculosis burden, the only susceptibility data that are likely to effect a change in therapy at the programmatic level are those for the detection of multidrug-resistant tuberculosis, for which the performance of the MODS assay is highly reliable and rapid (median time to the results of susceptibility testing, 7 days), providing clinically important information in a meaningful time frame. Although direct drug-susceptibility testing is conventionally viewed with suspicion — indirect testing of cultured strains is preferred — our data refute that view for rifampin and isoniazid in the MODS assay. However, susceptibility testing for *M. tuberculosis* is complex, and concordance among even regional laboratories performing gold-standard testing is particularly variable for ethambutol and streptomycin.[39] Our findings for these two drugs agreed with previous data for the MODS assay,[7] demonstrating insufficient concordance of the assay (at least in its current format) with gold standards to recommend usage.

Our study defines strengths and redundancies in the first-generation MODS assay and should enable the development of a streamlined, clinically useful method. The use of fewer wells per sample than we used — two wells with no drug (to ensure high specificity), one with rifampin (1 μg per milliliter), and one with isoniazid (0.4 μg per milliliter) — reduces costs by 40% but does not affect performance. The MODS assay is "laboratory freeware," not a commercial product or a kit. Any laboratory that is adequately biologically secured, has an incubator and a centrifuge, and is capable of

microscopy can safely perform MODS culture. All ingredients are available from major laboratory suppliers.

Downstream effects on patient care are the litmus test of the utility of a new method, and the added value will therefore depend on context and strategy for implementation. In countries where smear-negative tuberculosis is frequently diagnosed and treated empirically, the incremental benefit of MODS culture on case detection, as compared with the smear alone, would be less than that in Peru, where only 21% of treated cases are smear-negative and where MODS culture has recently been incorporated into Ministry of Health guidelines (www.minsa.gob.pe/normaslegales/2006/RM383-2006.pdf). However, the high specificity rate would save patients and society money by minimizing overtreatment, and the early detection and treatment of multidrug-resistant tuberculosis would interrupt transmission. Equity in the access to high-performance techniques to diagnose tuberculosis thus benefits both individual health[40,41] and public health. Despite the low cost per sample, in many resource-limited settings with a high tuberculosis burden, testing by the MODS assay of all patients with suspected tuberculosis (<5% of whom have culture-positive disease in Peru) would be a challenge financially and operationally. In our targeted, high-risk groups, only one MODS culture (collected in one visit) is required to achieve culture-positive rates of 20%, a good return on the investment. Programmatic studies are now needed to determine the optimal implementation strategy to maximize the effect and cost-effectiveness of this tool.

The MODS assay addresses two key gaps in resource-limited settings with a high tuberculosis burden: rapid, accurate detection of *M. tuberculosis* and simultaneous identification of multidrug-resistant tuberculosis. The use of culture-based diagnostic techniques for case detection may not be the future as envisaged by the International Union against Tuberculosis and Lung Disease[42]; promotion of such a strategy may conflict with the view that the scale-up of coverage and improvement of smear microscopy is currently a more important priority. However, we believe the MODS assay could now be implemented in settings in which smear microscopy is being optimally used and the augmentation of case detection is feasible and desirable.

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