Microscopic Observation Drug Susceptibility Assay for Tuberculosis Screening before Isoniazid Preventive Therapy in HIV-Infected Persons

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(See the editorial commentary by Minion and Pai, on pages 997–999.)

Background. Active tuberculosis (TB) must be excluded before initiating isoniazid preventive therapy (IPT) in persons infected with human immunodeficiency virus (HIV), but currently used screening strategies have poor sensitivity and specificity and high patient attrition rates. Liquid TB culture is now recommended for the detection of *Mycobacterium tuberculosis* in individuals suspected of having TB. This study compared the efficacy, effectiveness, and speed of the microscopic observation drug susceptibility (MODS) assay with currently used strategies for TB screening before IPT in HIV-infected persons.

Methods. A total of 471 HIV-infected IPT candidates at 3 hospitals in Lima, Peru, were enrolled in a prospective comparison of TB screening strategies, including laboratory, clinical, and radiographic assessments.

Results. Of 435 patients who provided 2 sputum samples, *M. tuberculosis* was detected in 27 (6.2%) by MODS culture, 22 (5.1%) by Lowenstein-Jensen culture, and 7 (1.6%) by smear. Of patients with any positive microbiological test result, a MODS culture was positive in 96% by 14 days and 100% by 21 days. The MODS culture simultaneously detected multidrug-resistant TB in 2 patients. Screening strategies involving combinations of clinical assessment, chest radiograph, and sputum smear were less effective than 2 liquid TB cultures in accurately diagnosing and excluding TB ($P < .01$). Screening strategies that included nonculture tests had poor sensitivity and specificity.

Conclusions. MODS culture identified and reliably excluded cases of pulmonary TB more accurately than other screening strategies, while providing results significantly faster than Lowenstein-Jensen culture. Streamlining of the ruling out of TB through the use of liquid culture–based strategies could help facilitate the massive upscaling of IPT required to reduce HIV and TB morbidity and mortality.

Tuberculosis (TB) is the most common serious opportunistic disease and a leading cause of death in persons infected with human immunodeficiency virus (HIV). An important source of new TB cases is the reactivation of latent TB infection (LTBI). This contribution has mushroomed in regions of high HIV prevalence because HIV greatly increases the risk for progression of LTBI to active TB disease [1]. This risk can be reduced by isoniazid preventive therapy (IPT), with benefits for both the individual [2, 3] and, by averting incident TB, public health [4, 5]. Effectiveness has been demonstrated in many populations [2, 6–9], although not all [10, 11], and it is generally accepted that the lifetime risk of reactivation TB can be reduced to ≤4% [12, 13].

Isoniazid alone is appropriate treatment for LTBI, but inadvertent isoniazid monotherapy for active TB is ineffective and leads to drug resistance. Therefore, the pathway to commencing IPT must reliably exclude active TB. Surprisingly, international consensus guidelines

on how this should be achieved are lacking. Challenges [3, 14] include procedural factors (the requirement for multiple investigations involving several visits leads to a patient attrition rate of 20% [15, 16]) and the poor sensitivity of conventional tests (clinical history [17], sputum smear, and chest radiography [16, 18, 19]) for detection of HIV-associated TB in which cough may be unproductive and chest radiography results normal. This bottleneck to successfully ruling out active TB is a significant obstacle to the up-scaling of IPT.

The microscopic observation drug susceptibility (MODS) assay is a low-cost liquid culture tool for TB culture and direct drug susceptibility testing (DST) developed for resource-limited areas. Diverse studies of the MODS assay [20–22] have demonstrated high specificity and sensitivity for diagnosis of TB and multidrug-resistant TB in symptomatic patients. Importantly, for TB to be ruled out, time to definitively negative culture with MODS is only 21 days; 99% of positive cultures are positive by 14 days.

These characteristics suggest that the MODS assay might be a useful tool for the pre-IPT TB screening of HIV-infected patients. We hypothesized that the high sensitivity of the MODS assay would negate the need for other investigations, and the rapid turnaround time would mitigate patient loss to follow-up and thus facilitate a greater flow of patients into IPT.

The Peruvian national strategy for ruling out TB before IPT involves clinical evaluation by a physician, chest radiography, and analysis of 2 sputum samples by Ziehl-Neelsen smear and solid media TB culture. In this study, we investigated how the culture of 1 or 2 sputum samples tested by the MODS assay alone would compare with the Peruvian and other TB screening strategies in terms of sensitivity, specificity, delay to diagnosis, and cost. The intended outcome was the delivery to policy-makers of robust comparative data to enable rational decision-making about resource allocation for ruling out TB.

METHODS

Study setting and population. HIV-infected candidates for IPT were consecutively recruited at the start of the workup for ruling out TB at 3 public hospitals in Lima, Peru, from February through December 2007. Exclusion criteria were age <15 years, current treatment with anti-TB agents, any IPT in the previous 12 months, and inability to give written informed consent. Patients were not paid for participation, but travel costs were fully reimbursed.

Field procedures. All patients underwent clinical evaluation by a physician to screen for pulmonary and extrapulmonary TB. Physicians referred to the study only those patients for whom the purpose of ruling out TB was the initiation of IPT. Although no enrolled patient was strongly suspected of having active TB, physicians were asked to assess the risk (none, low,

moderate, or high) of active TB relative to other IPT candidates on the basis of clinical history, symptoms, and CD4 cell count. For subsequent analyses, none or low were grouped as lower risk, and moderate or high were grouped as higher risk. After providing informed consent and undergoing interviews to record demographic, clinical, and socioeconomic data, patients were asked to provide 2 sputum samples (on different days) for the study in addition to the 2 samples required by the hospital laboratory for routine workup. Chest radiographs were interpreted by a single investigator masked to patient details, who was required to respond yes or no to the question, "Are there any findings suggestive of active TB for which further testing would be recommended prior to IPT?"

In Peru, no confirmation of LTBI (by tuberculin skin test [TST] or other means) is sought before IPT in HIV-infected persons. However, a positive TST result is used to identify IPT candidates in other settings, such as southern Africa [23], and was therefore included in this study. A TST (5 IU of purified protein derivative) was performed according to standard guidelines; a transverse induration of ⩾5 mm after 48–72 h was considered to be a positive result. A TST was not undertaken if the patient declined or reported having had a TST in the previous 6 months. Automated CD4 cell count was undertaken unless performed within the 6 months before enrollment, in which case this value was used.

Dedicated study personnel who rotated through all 3 study sites were trained collectively in data and specimen acquisition. Weekly team meetings ensured standardization of practices across the sites.

Laboratory procedures. Sputum samples were maintained at 4°C until same-day or next-day transport to the study laboratory, where they were digested and decontaminated by the standard *N*-acetyl-ʟ-cysteine-sodium hydroxide-sodium citrate method [24]. An aliquot was used for auramine smear microscopy and the remainder for parallel Lowenstein-Jensen (LJ) and MODS cultures, performed by 3 experienced laboratory technologists who were masked to patient details and the results of other tests. After inoculation, LJ slant cultures were incubated at 37°C and examined twice weekly from day 7 through day 60 [24]. Contaminated slant cultures were discarded, and additional decontamination and culture were undertaken using a stored portion of the original sample.

MODS culture. Five samples were each cultured in one 4-well column of a 24-well plate; a middle column served as a negative control. Each well contained 900 μL of decontaminated sputum, Middlebrook 7H9 broth, OADC (oleic acid dextrose catalase) growth supplement, and PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin) antibiotic supplement (full standard operating procedure available at http://www.modsperu.org). Plates were incubated at 37°C

Table 1. Participant Characteristics

Characteristics	Patients with no demonstrated TB[a] (n = 408)	Patients with active TB[b] (n = 27)	P[c]
Age, mean years ± SD	34.1 ± 10.7	34.7 ± 10.3	.78
Female sex	158 (39)	4 (15)	.01
Previous TB[d]	60 (15)	2 (7)	.29
Previous isoniazid preventive therapy[e]	58 (14)	4 (15)	.94
Recent TB contact[d]	125 (31)	8 (30)	.91
Positive tuberculin skin test result (≥5 mm)[f]	116 (32)	13 (59)	.008
CD4 cell count,[g] mean cells/μL ± SD	246 ± 190	164 ± 96	.06
Two or more symptoms (cough, fever, weight loss, night sweats) for ≥14 days[h,i]	60 (15)	8 (30)	.08

NOTE. Data are no (%) of patients, unless otherwise indicated. Nineteen patients who provided <2 sputum samples are excluded. Five patients were hospitalized at the time of enrollment; the remainder were ambulatory. SD, standard deviation; TB, tuberculosis.

[a] Defined as having only negative microbiologic test results.

[b] Defined as having at least 1 positive microbiologic test result.

[c] χ^2 test for categorical variables; t test for continuous variables.

[d] Self-reported; 1 nonresponse.

[e] Self-reported; 2 nonresponses.

[f] A total of 368 TB-negative patients and 22 TB-positive patients returned for reading of a tuberculin skin test result performed at the time of enrollment.

[g] A total of 375 TB-negative patients and 20 TB-positive patients had a documented CD4 cell count within 6 months of study enrollment.

[h] Self-reported by 404 TB-negative patients and 27 TB-positive patients.

[i] The positive predictive value (PPV) of having ≥2 of these symptoms for ≥14 days was 11.8%, the negative predictive value (NPV) was 94.8%, and the positive likelihood ratio (LR) was 1.7; for those with a CD4 cell count <200 cells/μL, the PPV was 10.0% ($P = .75$), whereas for those with a CD4 cell count ≥200 cells/μL, the PPV was 4.5% ($P = .62$). The PPV of having any of these symptoms for any period was 8.0%, the NPV was 97.7%, and the positive LR was 3.5. Three patients with active TB were asymptomatic. Because of the low PPVs, and because symptoms do not figure in Peruvian screening guidelines, no further evaluations of symptoms as a screening tool were undertaken.

and examined daily (weekdays only) from days 5 to 15, then on alternate days to day 25, under an inverted microscope at magnifications of ×40 and ×100. To minimize cross-contamination and occupational exposure, plates were sealed inside plastic bags and subsequently examined within the bag. If ≥2 CFU were detected in each of the 2 drug-free wells, the sample was considered to be positive [25–27]. Growth in a drug-containing well indicated resistance to that drug. If there was no evidence of growth by day 25, the culture was considered to be negative [20]. Fungal or bacterial contamination was recognized by rapid overgrowth or clouding; if detected, the stored portion of the original sample was decontaminated and cultured.

Definitions. A TB-positive patient was defined as one for whom *Mycobacterium tuberculosis* was detected by at least 1 microbiological test (auramine stain, LJ culture, or MODS culture). Indeterminate cultures were those that were persistently contaminated after repeat decontamination and culture. Patients with 2 smear- and culture-negative sputum samples were considered to be negative for TB.

Estimated initial costs of screening packages. Costs of the screening packages (combinations of screening tests) were estimated by including wholesale prices of test reagents, including

shipping, pro rata costs of local medical personnel time, and patient costs (median of transportation cost, personal lost income, and family lost income) as derived from a questionnaire. Costs for repeat culture due to contamination were also included. Downstream costs, overhead costs, and capital costs were not included. All costs were converted to 2007 US dollars and were based on current market costs for Lima public hospitals.

Data analysis. Data were analyzed with Stata statistical software, version 9 (Stata). To compare the equivalence of screening packages, McNemar's test was used with a significance level of $\alpha < .05$. Because some patients did not complete all strategies, because of either lack of follow-up or indeterminate results, separate tables of sensitivity and specificity were derived to reflect the results from an intent-to-screen perspective and from the subgroup for whom all test results were available. Characteristics of TB-confirmed and TB-excluded patients were compared using 2-sided t tests and χ^2 tests with a significance level of $\alpha < .05$.

Ethical review. Study protocol and consent and assent forms were approved by the ethics committees of Universidad Peruana Cayetano Heredia, Hospital Nacional Dos de Mayo,
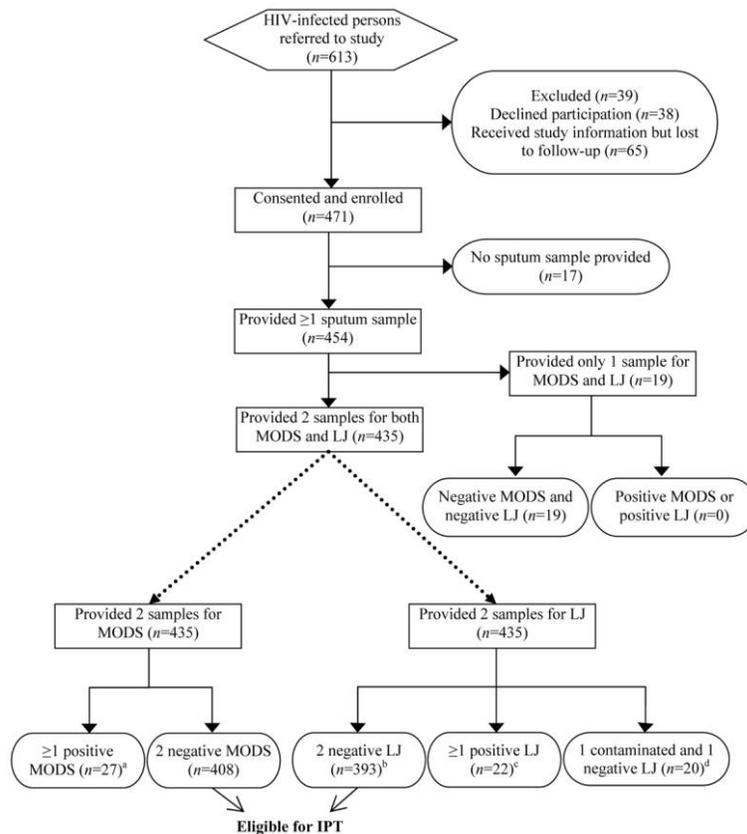
Figure 1. Patient flowchart for sputum testing by microscopic observation drug susceptibility (MODS) and Lowenstein-Jensen (LJ) cultures. Demographic data were available for 131 of the 178 patients referred to the study but ultimately not part of the inclusion group: 37.4% were women ($P = .97$, compared with participants), and the mean age ($\pm$ SD) was 36.7 ± 10.8 years ($P = .02$). CD4 cell count was available for 101 of the patients (mean $\pm$ SD, 261 ± 170 cells/μL; $P = .31$). HIV, human immunodeficiency virus; IPT, isoniazid preventive therapy. [a]Fifteen patients had 2 positive MODS cultures. Twelve patients had 1 positive and 1 negative MODS culture; of these, 3 patients had no positive LJ culture or auramine stain. [b]Three of the LJ culture–negative patients had at least 1 positive MODS culture. [c]Ten patients had 2 positive LJ cultures, 10 had 1 positive and 1 negative culture, and 2 had 1 positive and 1 contaminated culture. All 22 of the patients with $\geqslant$1 positive LJ culture had at least 1 positive MODS culture. [d]Two of the patients with 1 contaminated and 1 negative LJ culture had at least 1 positive MODS culture.

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RESULTS

Patients and samples. The characteristics of the population studied are given in Table 1 and the flowchart for sputum testing in Figure 1. Of 454 patients who provided at least 1 sputum sample, 129 (28%) reported undergoing highly active antiretroviral therapy at the time of enrollment. Nineteen patients provided only 1 sample for the study; their culture results were included only when the sample was used as the unit of analysis. Of 889 sputum samples, 44 (4.9%) were culture positive for *M. tuberculosis* by at least 1 method, of which 13 (30% of culture-positive samples) were auramine positive. By participant, 27 (6.2%) had at least 1 positive culture; 7 (26% of culture-positive patients) had a positive auramine smear microscopy result. No smear-positive sample was culture negative.

Table 2 indicates the comparative performances of culture methods. Sensitivities (95% confidence intervals) of the MODS and LJ cultures by sample were 95% (85%–99%) and 73% (57%–85%), respectively ($P = .02$, by McNemar's test), and by patient were 100% (87%–100%) and 81% (62%–94%), respectively ($P = .07$, by McNemar's test).

Contamination. Sixty-five samples (7.3%) were initially contaminated in MODS culture; subsequent decontamination yielded 64 definitively negative and 1 positive culture. With the LJ culture, 192 samples (22%) were initially contaminated ($P < .001$, vs MODS culture); subsequent decontamination yielded 166 negative, 4 positive, and 22 persistently contaminated, and thus indeterminate, cultures (2.5% of all samples) (Figure 1).

Added value of second culture. Of 27 patients with at least 1 positive MODS culture, 9 (33%) had only a positive second sample; 3 (11%) had only a positive first sample. Of 22 patients

Table 2. Comparison of the Microscopic Observation Drug Susceptibility (MODS) and Lowenstein-Jensen Culture Results for Tuberculosis (TB)

| MODS culture result | Lowenstein-Jensen culture result | | | |
| | Per sample | | Per patient[c] | |
	TB	Not TB	TB	Not TB
TB	30	12	22	5
Not TB	2[a]	845[b]	0	408[d]

[a] For the 2 samples that were positive on the Lowenstein-Jensen culture but negative on the MODS culture, the other sample from each patient was positive on the MODS culture; poor sample quality or division might explain the discrepancy.

[b] Includes 24 samples that were contaminated (indeterminate) in Lowenstein-Jensen media.

[c] Excludes 19 patients who provided only 1 sputum sample.

[d] Includes 20 patients who had 1 contaminated (indeterminate) and 1 negative culture in Lowenstein-Jensen media.

with at least 1 positive LJ culture, 7 (32%) had only a positive second sample; 5 (23%) had only a positive first sample.

Time to culture result, including delays due to contamination and additional culture. Median (interquartile range) time to culture positivity by the MODS and LJ cultures was 8 days (7–10 days) and 26 days (21–33 days), respectively (*P* < .001, by Wilcoxon test). Median time to definitive culture negativity by the MODS and LJ cultures was 28 days (26–31 days) and 63 days (61–66 days), respectively (*P* < .001, by Wilcoxon test). All TB-positive patients had a positive MODS culture within 21 days, and 96% had a positive MODS culture within 14 days.

Direct DST. Simultaneous MODS DST revealed that 6 of the 27 patients with culture-positive TB had strains resistant to isoniazid only, and 2 had strains resistant to both isoniazid and rifampin (multidrug resistant).

Clinical examination, chest radiography, and TST. Physician-judged clinical evaluation assigned 394 patients as being at lower risk and 60 patients as being at higher risk of active TB, of which 15 (3.8%) and 12 (20%), respectively, had culture-positive TB. The presence or absence of ≥2 constitutional symptoms did not usefully differentiate between those with and without active TB (Table 1). Although clinical outcomes were not a primary objective of this study, follow-up vital status data were available for 228 baseline TB-negative patients, representing 109,026 patient-days (297.12 patient-years); the remainder were lost to follow-up. Fourteen incident cases of TB were identified; mean time to diagnosis after a negative screen was 298 days (median, 178.5 days), and 79% (11 of 14 cases) occurred >3 months after screening.

The sensitivity of chest radiography for culture-positive TB in the 437 patients who completed radiographic examinations was 56% (95% confidence interval, 35%–75%). Culture-positive TB was demonstrated in 11 (3.1%) of 356 patients whose radiographic findings showed no evidence of active TB, and in

14 (17%) of 81 patients whose radiographic findings suggested further workup was needed before initiating IPT.

TST was performed in 445 patients, of whom 391 (88%) returned for reading in 2–3 days; TST results were positive in 130 (33% of returning patients).

Comparison of the MODS assay and other tests for ruling out TB. Performance characteristics of single tests and multitest screening packages and their associated costs are presented in Table 3. Comparative times to correct assignment as TB positive or TB negative are plotted in Figure 2.

Costs of initial diagnostic packages. The costs per person intended to screen are presented in Table 3. Specific costs and assumptions are presented in Tables 4 and 5.

DISCUSSION

The enormous disconnect between international policy recommendations of IPT for HIV-infected persons and the implementation of this proven intervention (reaching <0.08% of eligible people) owes much to the cumbersome nature of current strategies for ruling out TB and uncertainty about optimal approaches. This study demonstrates that culture-based approaches are the only way to deliver optimum TB screening. The MODS assay detected *M. tuberculosis* with greater sensitivity and speed and ruled out TB more quickly and with fewer indeterminate culture results than the LJ culture, which is consistent with data reported elsewhere [20, 28].

The strengths of this study derive from its prospective, real-world design and the fact that it was performed at multiple sites. All tests were intended for all patients, and the results were interpreted by staff masked to other results. Previous studies of TB screening strategies have used combinations of reported symptoms as a proxy for clinical assessment [29, 30]. In this study, referring clinicians directly assessed the global likelihood of active TB in each patient, similar to an approach previously described for individuals suspected of having TB [31]. This more accurately represents the real-world situation wherein a clinician decides on the basis of overall assessment, rather than a formalized score, whether a patient should continue on the path to IPT.

IPT candidates have a lower prevalence of TB than the groups previously studied with the MODS assay, who were largely patients suspected of having active TB [20–22]. Nevertheless, 6.2% of the patients in this study were found to have culture-positive TB; if IPT is initiated, these patients are at risk for developing drug-resistant TB [32]. Twenty of 27 culture-positive patients were smear negative and would have been administered IPT according to practices in which treatment decisions are made before obtaining culture results.

Wider use of DST features in the World Health Organization Strategic and Technical Advisory Group for Tuberculosis recommendations [33]. The MODS assay provides rapid, direct

Table 3. Tuberculosis Screening Package Performance Comparison, Ordered by Proportion of Patients Correctly Assigned

Package	Patients correctly assigned,[a] no (%) of patients (n = 435)	Completed package,[b] proportion (%) of patients		Proportion (%) of patients		Intent to screen[c] (n = 435) No of patients		Cost per person intended to screen,[e] 2007 US$
		Sensitivity of tests	Specificity of tests	Positive predictive value of package	Negative predictive value of package	Failed to complete package[d]	TB-positive cases missed	
2 MODS cultures	435 (100)	27/27 (100)	408/408 (100)	27/27 (100)	408/408 (100)	19	0	13.62
1 MODS culture	426 (97.9)	18/27 (67)	408/408 (100)	18/18 (100)	408/417 (97.8)	0	9	7.31
2 Smears	415 (95.4)	7/27 (26)	408/408 (100)	7/7 (100)	408/428 (95.3)	19	20	5.57
1 LJ culture	414 (95.2)	15/25 (60)	399/399 (100)	15/15 (100)	408/420 (97.1)	11	12	6.39
2 LJ cultures	412 (94.7)	22/25 (88)	390/390 (100)	22/22 (100)	408/413 (98.8)	39	5	11.77
No screening (all would get IPT)	408 (93.8)	0/27 (0)	408/408 (100)	NA	408/435 (93.8)	0	27	0.00
Clinical examination and 2 MODS cultures	393 (90.3)	27/27 (100)	366/408 (89.7)	27/69 (39.1)	366/366 (100)	19	0	14.14
Clinical examination and 2 smears	380 (87.4)	14/27 (52)	366/408 (89.7)	14/56 (25)	366/379 (96.6)	19	13	6.09
Clinical examination	378 (86.9)	12/27 (44)	366/408 (89.7)	12/54 (22)	366/381 (96.1)	0	15	1.52
Clinical examination, 2 smears, and 2 LJ cultures	371 (85.3)	23/25 (92)	348/390 (89.2)	23/65 (35)	366/370 (98.9)	39	4	14.86
Chest radiograph	351 (80.7)	14/25 (56)	337/399 (84.5)	14/76 (18)	346/359 (96.4)	17	13	9.17
Southern Africa algorithm[f] (430 total patients)	350 (81.4)	11/21 (52)	338/358 (94.4)	12/33 (36)	382/397 (96.2)	87	15	17.03
Chest radiograph and clinical examination	330 (75.9)	18/25 (72)	310/399 (77.7)	20/111 (18)	317/324 (97.8)	17	7	9.70
Chest radiograph, clinical examination, and 2 smears	330 (75.9)	18/25 (72)	310/399 (77.7)	20/111 (18)	317/324 (97.8)	30	7	13.26
Chest radiograph, clinical examination, 2 smears, and 2 LJ cultures	320 (73.6)	22/23 (96)	295/381 (77.4)	25/116 (22)	317/319 (99.4)	50	2	22.03

NOTE. Tuberculosis (TB)–positive cases were defined as patients having any positive microbiological test result. Patients who provided <2 sputum samples did not have an adequate culture reference standard and thus were excluded from the table. Positive clinical examination result indicates a higher risk of active TB, according to the referring clinician. Positive chest radiography results indicate findings consistent with active TB for which further testing would be recommended before isoniazid preventive therapy. Southern Africa algorithm indicates preliminary screening by tuberculin skin test (TST); then, for only those with positive TST results, clinical examination, chest radiography, 2 smears, and Lowenstein-Jensen (LJ) cultures. IPT, isoniazid preventive therapy; MODS, microscopic observation drug susceptibility; NA, not applicable.

[a] Indicates those correctly assigned as TB positive or TB negative by the package, with the reference standard being any positive microbiological test result. Patients who failed to complete all components of a package were considered not correctly assigned, unless at least 1 component test result was positive in the context of a positive culture result.

[b] Excludes patients who did not undergo ≥1 components of the package (eg, chest radiography) or who had 1 contaminated (indeterminate) and 1 negative LJ culture; denominators vary according to completion rate of the package. This is the "as screened" analysis, which provides efficacy data for completed packages.

[c] Within a package, a positive result for any component was considered to be a positive result for the package. This intention-to-screen analysis describes overall performance in all those who entered into screening and incorporates the effect of loss to follow-up.

[d] This is the only column that includes the 19 patients who provided only 1 sputum sample; these patients were considered to have failed to complete all packages involving 2 sputum samples.

[e] This value was attained by summing the cost of diagnosis and patient costs from Tables 4 and 5, then dividing the result by the 435 patients presented in Table 3. The median of the patient costs, including transport, was used.

[f] Five patients did not undergo TST and thus were excluded from analysis of this package.
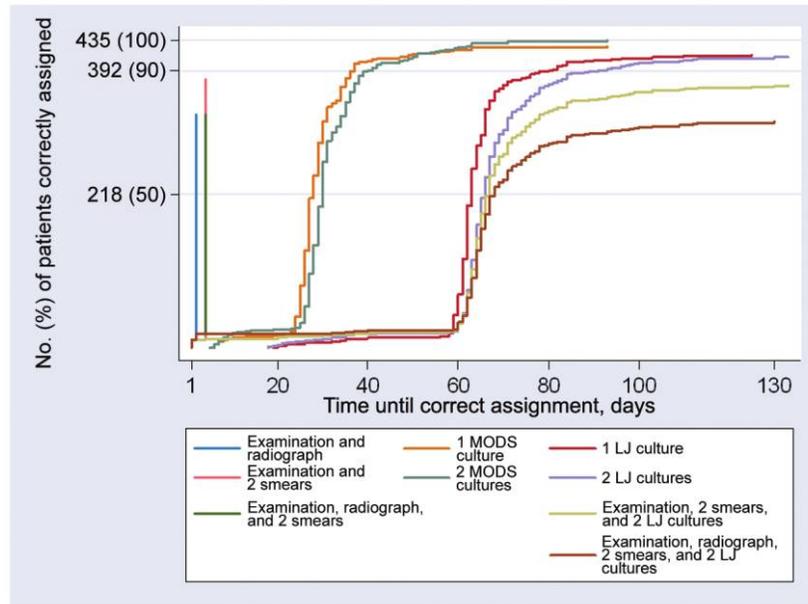
Figure 2. Time to correct assignment of patients by the various screening packages. This graph represents the time until the 435 patients who provided 2 sputum samples would have been correctly assigned as tuberculosis (TB) positive or negative by the various screening packages. Each package is represented by a distinct line, with the maximum height on the *y*-axis signifying the number of patients correctly assigned by that package. A patient was considered correctly assigned if and when (1) any component of the screening package was positive and any microbiologic test result was positive or (2) all components of the screening package were negative and all microbiologic test results were negative. Patients who did not complete all components of a package were considered to be not correctly assigned, unless at least 1 component test result was positive in the context of a positive microbiologic test result. Packages delivering the best performance in correct patient assignment reach furthest up the *y*-axis, whereas the speed with which results are available is reflected on the *x*-axis. An ideal package would be represented by a straight vertical line that ended in the top left-hand corner. Reculturing times for contaminated samples are included. LJ, Lowenstein-Jensen; MODS, microscopic observation drug susceptibility.

DST; results for the 2 patients with multidrug-resistant TB were available in 6 and 9 days.

We previously demonstrated limited incremental value of a second MODS culture in individuals suspected of having TB and hospitalized, HIV-infected persons [20]. We predicted a similar finding in the present study in a population with relatively low suspicion of TB. However, 50% more cases were detected with a second culture. This finding likely reflects the pauci-bacillary nature of TB in HIV-infected persons with minimal symptoms and the quality of sputum obtained. The increased yield of second sputum samples might be attributable to recruitment and follow-up practices; second samples were more likely than first samples to be provided in the early morning. Nevertheless, a single liquid culture still correctly assigned more people as TB positive or TB negative than any screening package that did not include the MODS assay.

Deciding on the most important parameter when comparing screening packages is not straightforward. Specificity indicates how well noncases are identified but gives no information about cases misclassified as noncases (having no screening at all and giving all candidates IPT has a specificity of 100%). Negative predictive value describes how much confidence to put in a noncase result but is an unstable parameter modified by local prevalence of cases. To capture the overall performance, we compared screening packages by determining the number of patients who would have been correctly assigned to IPT or active TB treatment. Interestingly, all currently recommended screening packages would have correctly assigned fewer patients than having no screening at all (ie, assigning all patients referred to the study as TB negative); only the laboratory-based strategies correctly assigned more patients than no testing at all

As described elsewhere [16, 18], chest radiography appears to be a particularly costly and ineffective tool; low sensitivity for detecting culture-positive cases indicates that a negative radiography result is not sufficient grounds to start IPT. These data suggest, perhaps controversially, that chest radiography could be dropped from strategies for ruling out TB in situations where only pauci-symptomatic patients enter the algorithm.

Attrition rates—assessed by how many patients failed to undergo chest radiography or provide a second sputum sample— were low. Attrition is likely to be higher in nonstudy situations

Table 4. Estimated Costs of Initial Screening

This table is available in its entirety in the online version of *Clinical Infectious Diseases*

Table 5. Package Costs for 435 Patients

This table is available in its entirety in the online version of *Clinical Infectious Diseases*

where patients assume transportation and test costs and follow-up supervision is less rigorous.

The 19 patients who did not provide a second sputum sample were excluded from analyses of strategies because all had a single negative culture result, which is insufficient for definitive classification as TB negative. However, these would be considered failures of the "2 MODS" strategy.

We defined active TB as any positive microbiological result. Because the primary purpose was ruling out TB, positive results from any test with proven high specificity, including those under evaluation for this new indication (such as the MODS assay) cannot be ignored, particularly when the test is known to be more sensitive than existing reference standards [20–22]. High specificity and infrequent cross-contamination of the MODS assay have been previously demonstrated, which somewhat attenuates the threat of incorporation bias [20].

Of 14 baseline TB-negative patients subsequently diagnosed as having TB, only 3 occurred within 3 months of a negative screen. Although IPT initiation and monitoring were beyond the scope of the study, it was determined that many patients had not initiated IPT or had not been adherent. Though the 11 later diagnoses almost certainly represent either newly acquired disease or reactivation of pre-existing LTBI in patients truly disease free at baseline, it is challenging to determine whether this also applies to the 3 early cases or whether their disease represents progression of previously undetected active TB and thus a false-negative screen.

The high rate of initially contaminated LJ cultures is not easily explained; when the same sputum decontaminate is used for both the LJ and MODS cultures, as in this study, contamination in the LJ culture usually only slightly exceeds that in the MODS culture. Nevertheless, after additional decontamination only 2.5% of all samples yielded an indeterminate LJ culture; thus, although turnaround times were prolonged the detection performance of the LJ culture was not significantly compromised.

An important consideration is the assumed validity of our definition of non-TB cases (negative microbiologic test results for 2 sputum samples). Obvious weaknesses are that poor sputum sample quality can compromise smear and culture performance and that sputum testing cannot detect isolated extrapulmonary TB. False-positive MODS cultures are extremely unusual [28]; thus, specificity and positive predictive value are high.

IPT has the potential to save millions of lives and contribute importantly to TB control in regions with high HIV burden. Ruling out TB is a major bottleneck to implementation. Strategies to streamline and expedite this process could get millions of eligible HIV-infected persons the IPT they require. Increasing the proportion of eligible patients who are screened from the current <0.1% to just 10% could avert >1 million new cases of TB.

On the basis of the results of this study, we propose that liquid culture of 2 sputum samples alone can be used as an effective screening strategy for pulmonary TB before IPT in HIV-infected persons. The worldwide movement toward greater use of liquid culture and TB laboratory capacity building in resource-limited settings provides an opportunity for roll-out that could translate this research into large-scale practice. Clinical history and examination can provide additional information about the risk of extrapulmonary TB, but they are not a useful filter for pulmonary TB screening. Future studies can address the utility of improved sputum collection, perhaps after instruction [34], as a means to improve the sensitivity of a single culture in this population.

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