Microscopic-observation drug susceptibility and thin layer agar assays for the detection of drug resistant tuberculosis: a systematic review and meta-analysis

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Summary

Background **Simple, rapid, and affordable tests are needed to detect drug resistance in *Mycobacterium tuberculosis*. We did a systematic review and meta-analysis to investigate the accuracy of microscopic-observation drug susceptibility (MODS) and thin layer agar (TLA) assays for rapid screening of patients at risk of drug-resistant tuberculosis.**

Methods **In accordance with protocols and methods recommended by the Cochrane Diagnostic Test Accuracy Working Group, we systematically searched PubMed, Embase, and Biosis for reports published between January, 1990, and February, 2009. We included studies investigating detection of drug resistance in *M tuberculosis* with the MODS or TLA assay, and in which an accepted reference standard was used. Data extracted from the studies were combined by use of bivariate random-effects regression models and hierarchical summary receiver operating characteristic curves to estimate sensitivity and specificity for detection of resistance to specific drugs.**

Findings **We identified 12 studies, of which nine investigated the MODS assay and three investigated the TLA assay. For the MODS assay of rifampicin resistance, pooled estimates were 98·0% (95% CI 94·5–99·3) for sensitivity and 99·4% (95·7–99·9) for specificity. For the MODS assay of isoniazid resistance with a 0·1 μg/mL cutoff, pooled sensitivity was 97·7% (94·4–99·1) and pooled specificity was 95·8% (88·1–98·6), but with a 0·4 μg/mL cutoff, sensitivity decreased to 90·0% (84·5–93·7) and specificity increased to 98·6% (96·9–99·4). All assessments of rifampicin and isoniazid resistance with the TLA assay yielded 100% accuracy. Mean turnaround time was 9·9 days (95% CI 4·1–15·8) for the MODS assay and 11·1 days (10·1–12·0) for the TLA assay.**

Interpretation **MODS and TLA assays are inexpensive, rapid alternatives to conventional methods for drug susceptibility testing of *M tuberculosis*. Our data and expert opinion informed WHO's recommendation for use of selected non-commercial drug susceptibility tests, including MODS, as an interim solution until capacity for genotypic or automated liquid culture drug susceptibility testing is developed.**

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Introduction

The growing problem of drug resistance in *Mycobacterium tuberculosis* is accompanied by increasing demand for quick, cheap, and easy techniques to detect resistance.[1] Patients infected with multidrug resistant (MDR) or extensively drug resistant (XDR) strains of *M tuberculosis* need treatment regimens that include second-line drugs in addition to extended treatment duration.[2] Even when treated appropriately, MDR and XDR tuberculosis have substantially worse outcomes[3] and longer infectious periods than does drug-susceptible tuberculosis.[4] Therefore, prevention of the spread of MDR and XDR tuberculosis is important, for which timely identification of such cases is the first and most crucial step.

Several methods to detect drug resistance are available, but none clearly satisfies the demands of quick, cheap, and easy. Traditional agar-based methods can take months for results. Commercial drug susceptibility testing with liquid culture decreases turnaround times but requires expensive equipment. Molecular detection of gene mutations associated with drug resistance has also been developed, with variable sensitivity reported especially for in-house methods.[5] Commercial versions of line-probe assays have high accuracy[6,7] and were recently endorsed by WHO for rapid screening of MDR tuberculosis.[8] However, the expertise needed for a laboratory to offer molecular diagnostics is too great for these tests to be implemented in many resource-constrained settings with poor laboratory infrastructure.

Although liquid cultures and line-probe assays have been endorsed by WHO and phased implementation is underway in many countries, interim measures are needed to meet the needs of low-income settings with high rates of MDR and XDR tuberculosis. Non-commercial techniques to test drug susceptibility with inexpensive and widely available laboratory equipment and supplies—such as microscopic-observation drug susceptibilty (MODS) and thin layer agar (TLA) assays—could be implemented in these settings with minimum cost and training. In both MODS and TLA testing, drug-free and drug-containing media (liquid for MODS, solid for TLA) are inoculated with specimens from patients, and cultures are microscopically examined for early growth or microcolonies.[9,10] Growth of *M tuberculosis* in

or on drug-free media indicates a positive culture, whereas growth of *M tuberculosis* in or on both drug-free and drug-containing media indicates resistance.

We did a systematic review of published reports and a meta-analysis of studies examining the diagnostic accuracy, performance (including contamination rate and turnaround time), and outcomes important to patients of MODS and TLA assays for the detection of drug resistance in *M tuberculosis*. We followed a standard protocol for systematic reviews and meta-analyses,[11] and used methods recommended by the Cochrane Diagnostic Test Accuracy Working Group.[12]

Methods

Search strategy and study selection

We searched PubMed, Embase, and Biosis for reports published between January, 1990, and February, 2009 (inclusive). The first search used the terms tuberculosis[mesh] OR mycobacter*[ti] OR acid-fast[ti] OR tuberculous[ti] and the second search used the terms MODS[tw] OR "microscopic-observation"[tiab] OR "drug-susceptibility"[ti] OR (drug[ti] AND susceptibility[ti]) OR microcolony[tiab] OR (liquid[ti] AND culture[ti]) OR thin-layer[tiab] OR (thin[tiab] AND layer[tiab] AND agar[tiab]) OR agar[tiab] OR (mycobacterial[ti] AND culture[ti]); results from the first and second searches were combined with the Boolean operator AND so that all studies identified were listed in the results of both the first and second searches. All database searches were done independently by an experienced librarian and JM. We restricted the search to reports published in English, French, or Spanish. Studies were selected independently by two reviewers (JM and EL) and disagreements were resolved by consensus. Titles and abstracts were screened for relevance and any citations identified by either reviewer were selected for full-text review. Articles retrieved for full-text review and full reasons for exclusion are available from the authors. Reference lists from selected studies were hand searched and experts and test developers were contacted to identify additional studies.

Predetermined eligibility criteria for studies included in the primary analysis were assessment of the MODS or TLA assay for drug susceptibility of *M tuberculosis*, and use of an accepted reference standard. Accepted reference standards included indirect proportion methods (eg, a sensitive isolate has <1% resistant population whereas a resistant isolate has >1%), absolute concentration, resistance ratio, commercial liquid systems (BACTEC 460, Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA; MGIT 960, Becton Dickinson Diagnostic Instrument Systems; MB/BacT, Organon Teknika, Turnhout, Belgium), or microdilution methods. We included studies in which either the specimens from patients were used for direct inoculation or previously cultured isolates were used for indirect inoculation. Studies in which outcomes other than accuracy were measured, including outcomes important to patients, were reviewed and summarised narratively.

Assessment of study quality

We used the QUADAS criteria[13] for assessment of quality of diagnostic accuracy in studies to assess quality characteristics that were judged to be important for this review: masked interpretation of the test result with reference standard results and vice-versa; complete verification of test results with the reference standard; recruitment of patients or collection of specimens either consecutively or randomly; and cross-sectional study design (not case-control).

Data extraction

We created and piloted a data extraction form with a subset of eligible studies and used experience gained from the pilot study to finalise the extraction form. All studies included in the final review were extracted independently by two reviewers (JM and EL) and any disagreements were resolved by consensus. Data were extracted for every drug tested to construct two-by-two tables of true positive, false positive, false negative, and true negative values. We defined true positives as isolates identified as drug resistant by the MODS or TLA assay and the reference method. False positives were isolates identified as resistant by the MODS or TLA assay, but susceptible by the reference method. False negatives were isolates identified as susceptible on MODS or TLA assay, but resistant in the reference method. True negatives were isolates identified as susceptible by the MODS or TLA assay and the reference method.

Statistical analysis

We calculated the sensitivity and specificity for every drug tested. Results are presented separately by index test (MODS or TLA) for every drug tested. Subgroup analysis was done according to the drug concentration used to define the resistance cutoff. For sensitivity and specificity subgroup estimates, pooled with a random-effects model, we regarded subgroups with overlapping 95% CIs to be homogeneous groups and combined these groups for the primary analysis. Otherwise results are presented separately for different cutoffs. Subgroup analysis was also done with exclusion of studies with substantial protocol deviations or using microdilution reference standards. We also planned a subgroup analysis by the type of specimen used (ie, direct samples from patients or indirect isolates).

Other outcomes extracted included the following: turnaround time, defined as the time from specimen receipt or processing in the laboratory to availability of results in the laboratory; contamination rate, defined as the proportion of specimens contaminated with bacterial or fungal growth on first inoculation or appropriate assessment of cross-contamination; cost estimates, which include costs for reagents, supplies, equipment,

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labour, overheads, or other justified related costs; and outcomes important to patients.

Data were analysed with Stata/IC (version 11.0). Forest plots of sensitivity and specificity estimates and their 95% CIs were constructed from every study with MetaDiSc software (version 1.4) and by use of exact methods for proportions.[14] Sensitivity and specificity estimates tend to be correlated and vary according to thresholds (either explicit or implicit cutoff values determining whether results are positive or negative), so we analysed hierarchical summary receiver operating characteristic (HSROC) curves to explore the effect of the thresholds and produce an overall summary of test accuracy.[15–17] Accuracy measures were pooled by use of bivariate random-effects regression models,[18] with the user-written program metandi in Stata.[19] Heterogeneity of accuracy estimates was assessed by I^2 statistic.[20] Subgroups with fewer than four studies were combined by use of univariate random-effects models because bivariate random-effects regression models do not converge with small numbers of studies. Contamination rates were combined by use of weighted means and turnaround times were combined by use of simple means.

Role of the funding source

WHO had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The authors had full access to all study data and are solely responsible for the decision to submit for publication.

Results

We identified 2072 citations, of which 1406 unique articles remained after exclusion of duplicate articles, and 54 remained after screening of titles and abstracts (figure 1). These articles were retrieved for full-text review, of which 12 were eligible for inclusion in the primary analysis: nine of MODS testing[21–29] and three of the TLA

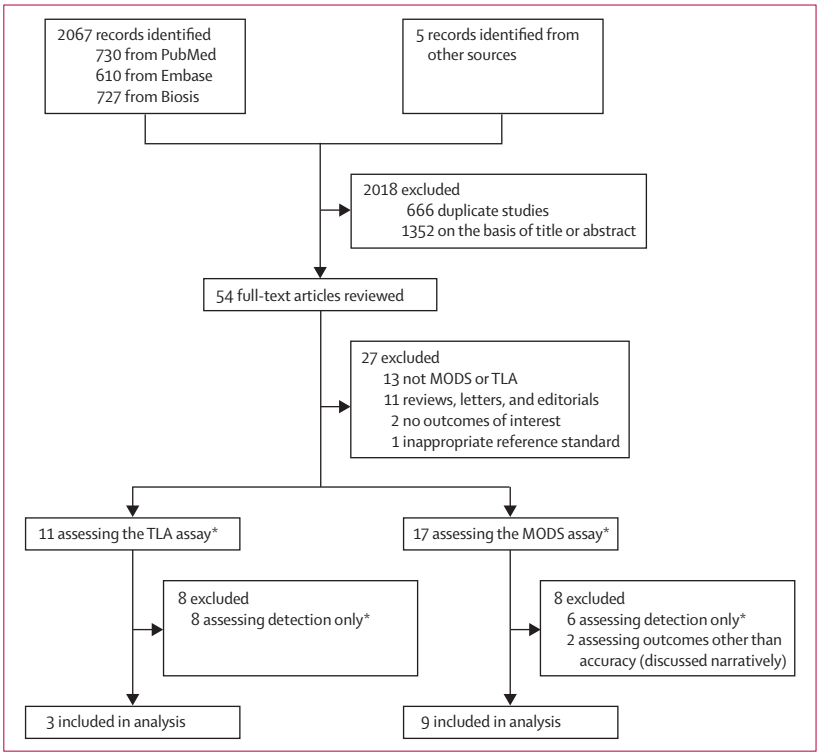

Figure 1: **Study selection**
MODS=microscopic-observation drug susceptibility. TLA=thin layer agar. *One study assed both the MODS and TLA assays.

	Samples	Country	Drugs tested	Reference	Inoculation*	Smear positive	Sputum	HIV positive
Microscopic-observation drug susceptibility assay								
Moore et al (2004)[21]	207	Peru	Rifampicin, isoniazid, ethambutol, streptomycin	MABA	Direct	NS	NS	NS
Caviedes et al (2000)[22]	88	Peru	Rifampicin, isoniazid	MABA	Direct	100%	100%	NS
Devasia et al (2009)[23]	239	USA	Ofloxacin	Proportion method	Indirect	NA	NA	NA
Ejigu et al (2008)[24]	58	Ethiopia	Rifampicin, isoniazid	MGIT 960	Direct	100%	100%	NS
Mello et al (2007)[25]†	180	Brazil	Rifampicin, isoniazid	Proportion method	Direct	NS	100%	NS
Mengatto et al (2006)[26]	64	Argentina	Rifampicin, isoniazid, ethambutol, streptomycin	Proportion method	Indirect	NA	NA	NA
Moore et al (2006)[27]	338	Peru	Rifampicin, isoniazid, ethambutol, streptomycin	Proportion method	Direct	5·9%	100%	7·9%
Park et al (2002)[28]	53	USA	Rifampicin, isoniazid, ethambutol, streptomycin	Proportion method	Indirect	NA	NA	NA
Shiferaw et al (2007)[29]	247	Ethiopia	Rifampicin, isoniazid	Proportion method	Direct	100%	100%	NS
Thin layer agar assay								
Martin et al (2009)[30]	147	Belgium	Rifampicin, ofloxacin, kanamycin	Proportion method	Indirect	NA	NA	NA
Robledo et al (2008)[31]	95	Columbia	Rifampicin, isoniazid	Proportion method	Direct	100%	100%	NS
Schaberg et al (1995)[32]	197	Germany	Rifampicin, isoniazid, ethambutol, streptomycin, pyrazinamide	Proportion method	Direct	48%	100%	1%

MABA=microplate alamar blue assay. NS=not specified (studies using direct inocula). NA=not applicable (studies using indirect inocula). *Direct inoculation refers to direct application of a specimen from a patient (processed or unprocessed) to drug-containing and drug-free media; indirect inoculation refers to application of a previously isolated strain of *Mycobacterium tuberculosis* to drug-containing and drug-free media. †Resistance defined as growth in drug-containing wells on day 14 after growth was detected in drug-free wells, which is opposed to the standard procedure to detect the presence of growth in drug-containing wells on the same day as growth is detected in drug-free wells.

Table 1: **Characteristics of studies included**

assay[30–32] (table 1; see webappendix for primary accuracy data from included studies). We identified two additional studies that did not measure accuracy, and so were not included in the main meta-analysis, but reported on other outcomes of interest.[33,34] All but one study presented results for resistance to rifampicin and all but two presented results for resistance to isoniazid. Data were also available from some studies for resistance to ethambutol, streptomycin, ofloxacin, kanamycin, and pyrazinamide. Three MODS studies and one TLA study used previously cultured isolates (indirect inoculates) and the remainder used clinical specimens (direct inoculates) for resistance testing. Four studies (three MODS and one TLA) used only smear-positive specimens, and seven studies (five MODS and two TLA) used only sputum specimens. Only two studies, one of each assay type, provided information on the HIV status of patients providing clinical specimens for testing. All studies verified their complete study sample with a reference standard and eight of 12 had a cross-sectional design (table 2). Half the studies used either consecutive or random samples, and half stated that test interpretation was masked with respect to the reference standard results. Six studies were done prospectively, three were done retrospectively, and three did not clearly report type of recruitment.

	Microscopic-observation drug susceptibility assay (n=9)	Thin layer agar assay (n=3)
Recruitment of specimens or patients		
Prospective	4	2
Retrospective	2	1
Unclear	3	0
Study design		
Cross-sectional	6	2
Unclear	3	1
Sampling		
Consecutive or random	5	1
Convenience	3	0
Unclear	1	2
Verification with accepted reference		
Complete	9	3
Masked interpretation of index and use of reference test		
Yes	5	2
Unclear	4	1

Table 2: **Study quality**

Sensitivity and specificity estimates for assessment of rifampicin resistance are shown for the MODS assay (figure 2) and TLA assay (figure 3). The accuracy of studies that used a 1 μg/mL concentration cutoff did not differ from those that used 2 μg/mL (data not shown) so results for all rifampicin assessments were combined. Estimates of sensitivity and specificity are also presented for resistance to isoniazid with the MODS assay (figure 4) and TLA assay (figure 5). For the MODS assay, data were stratified by drug concentrations of 0·1 μg/mL and 0·4 μg/mL; data differed significantly between these cutoff values so all results for the MODS assay of isoniazid resistance were stratified accordingly. Several studies tested both drug concentrations and so have two sets of results. For the TLA assay, studies used drug concentrations of 0·2 μg/mL and 0·25 μg/mL isoniazid. Estimates from the MODS assay had less variability with rifampicin than with isoniazid for sensitivity (range 92–100% *vs* 82–100%) and specificity (83–100% *vs* 78–100%). Sensitivity and specificity estimates for resistance to rifampicin and isoniazid with the TLA assay were all 100%, but few studies were included (three for rifampicin, two for isoniazid).

For drugs tested in only one study for each type of assay, both the MODS[23] and TLA[30] assays reported resistance to ofloxacin with 100% accuracy (table 3). Resistance to each of ethambutol, streptomycin, pyrazinamide, and kanamycin was also assessed with the TLA assay which seemed reliable but few data were available (table 3). For drugs tested in more than one study, we pooled estimates for sensitivity and specificity (table 4). For the MODS assay, sensitivity and specificity estimates were highest for resistance to rifampicin. Use of the 0·1 μg/mL isoniazid cutoff was associated with significantly higher sensitivity, but lower specificity (not significant), than was the

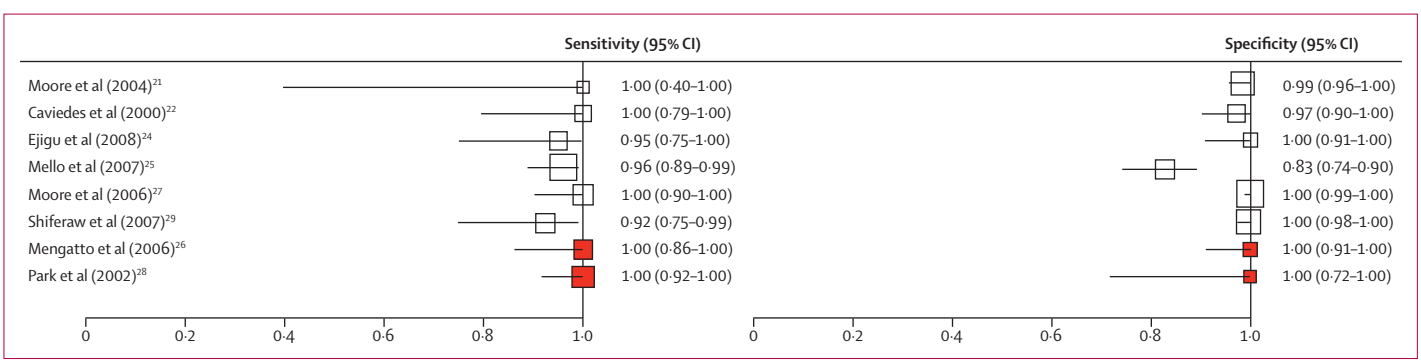

Figure 2: **Forest plot of accuracy of the microscopic-observation drug susceptibility assay for rifampicin resistance**
Open squares represent studies using direct inoculation with specimens from patients; red squares represent studies using indirect inoculation with isolates. Size of the square is proportionate to the size of the study.

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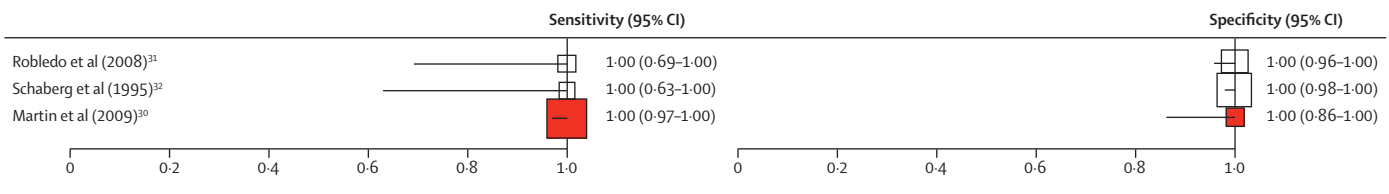

Figure 3: **Forest plot of accuracy of the thin layer agar assay for detection of rifampicin resistance**
Open squares represent studies using direct inoculation with specimens from patients; red squares represent studies using indirect inoculation with isolates. Size of the square is proportionate to the size of the study.

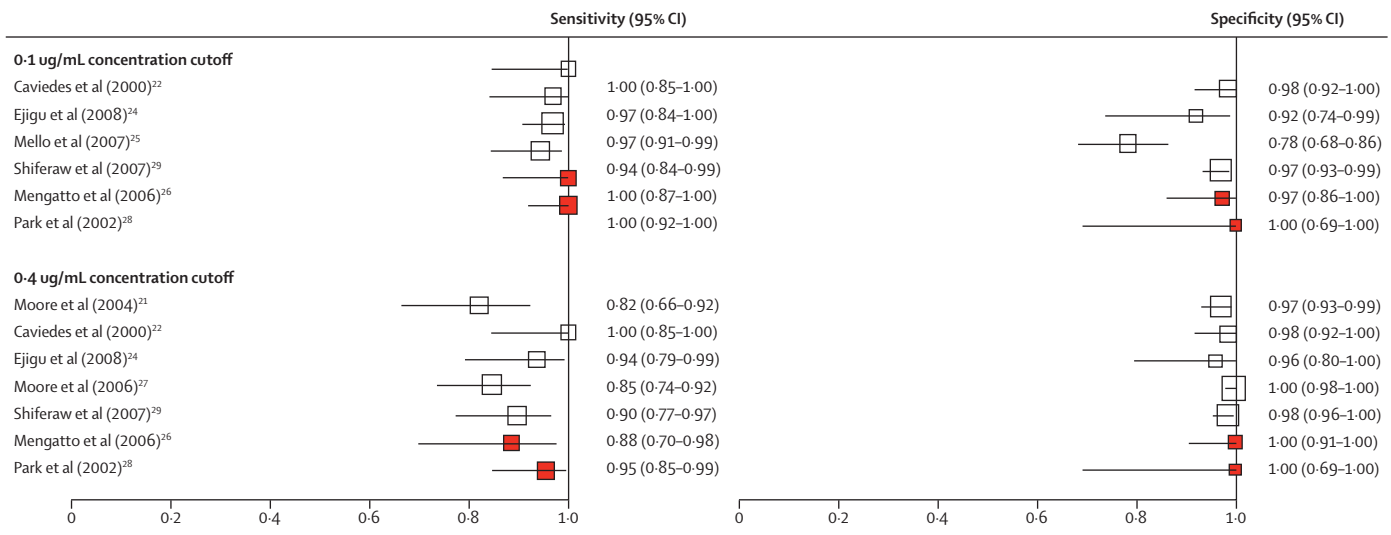

Figure 4: **Forest plot of accuracy of the microscopic-observation drug susceptibility assay for detection of isoniazid resistance**
Open squares represent studies using direct inoculation with specimens from patients; red squares represent studies using indirect inoculation with isolates. Size of the square is proportionate to the size of the study.

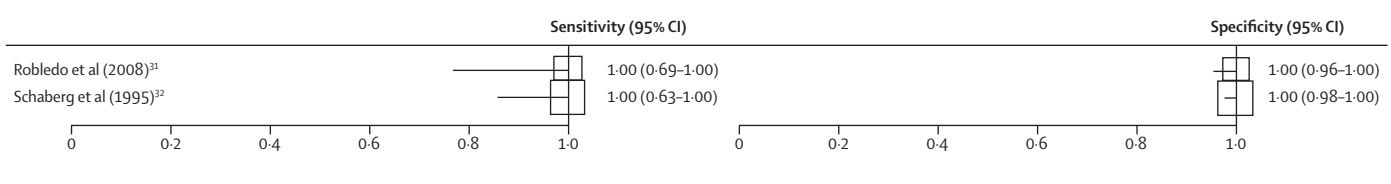

Figure 5: **Forest plot of accuracy of the thin layer agar assay for detection of isoniazid resistance**
Both studies used direct inoculation with specimens from patients. Size of the square is proportionate to the size of the study.

	Study	Specimen	True positive	False positive	False negative	True negative	Sensitivity (95% CI)	Specificity (95% CI)
Microscopic-observation drug susceptibility assay								
Ofloxacin	Devasia et al (2009)[23]	Indirect	6	0	0	233	100% (54·1–100)	100% (98·4–100)
Thin layer agar assay								
Ethambutol	Shaberg et al (1995)[32]	Direct	5	1	0	191	100% (47·8–100)	99·5% (97·1–100)
Streptomycin	Shaberg et al (1995)[32]	Direct	21	0	0	176	100% (83·9–100)	100% (97·9–100)
Pyrazinamide	Schaberg et al (1995)[32]	Direct	6	0	0	189	100% (54·1–100)	100% (98·1–100)
Ofloxacin	Martin et al (2009)[30]	Indirect	39	0	0	95	100% (91·0–100)	100% (96·2–100)
Kanamycin	Martin et al (2009)[30]	Indirect	67	1	0	77	100% (94·6–100)	98·7% (93·1–100)

Table 3: **Drugs assessed by single studies**

	Number of studies	Pooled sensitivity (95% CI)	I^2* (p value)	Pooled specificity (95% CI)	I^2* (p value)
All included studies					
Microscopic-observation drug susceptibility assay					
1 or 2 µg/mL rifampicin	8	98·0% (94·5–99·3)	0·0% (0·86)	99·4% (95·7–99·9)	68·5% (0·002)
0·1 µg/mL isoniazid	6	97·7% (94·4–99·1)	22·4% (0·27)	95·8% (88·1–98·6)	84·4% (<0·0001)
0·4 µg/mL isoniazid	7	90·0% (84·5–93·7)	45·9% (0·09)	98·6% (96·9–99·4)	18·8% (0·29)
2·5 µg/mL ethambutol	4	60·0% (47·3–71·6)	46·6% (0·13)	95·4% (83·1–98·9)	80·6% (0·001)
2 µg/mL streptomycin†	3	70% (62–78)	58·0% (0·09)	99% (97–100)	69·3% (0·54)
6 µg/mL streptomycin†	3	44% (32–57)	69·3% (0·04)	99% (97–100)	0% (0·45)
Thin layer agar assay					
1 µg/mL rifampicin†	3	100% (97–100)	0% (1·0)	100% (99–100)	0% (1·0)
0·2 or 0·25 µg/mL isoniazid†	2	100% (91–100)	0% (1·0)	100% (99–100)	0% (1·0)
Studies with stringent exclusion criteria applied‡					
Microscopic-observation drug susceptibility assay					
1 or 2 µg/mL rifampicin	5	98·7% (89·4–99·9)	45·4% (0·12)	99·9% (95·8–100)	0% (0·73)
0·1 µg/mL isoniazid	4	97·6% (90·9–99·4)	41·6% (0·16)	96·6% (93·4–98·3)	0% (0·57)
0·4 µg/mL isoniazid	5	90·2% (84·4–93·9)	5·9% (0·37)	99·0% (97·3–99·6)	2·4% (0·39)
2·5 µg/mL ethambutol†	3	67% (55–77)	0% (0·81)	97% (95–99)	91·2% (<0·0001)
6 µg/mL streptomycin†	2	45% (32–58)	84·5% (0·01)	100% (94–100)	0% (1·0)

*I^2 statistic measures the percentage of total variation across studies due to heterogeneity. †Pooled estimates calculated with the univariate random-effects model because too few studies were available for the bivariate random-effects model to converge. ‡Only studies in which estimates were affected by stringent exclusion criteria are listed.

Table 4: **Pooled accuracy estimates, stratified by test and drug**

	Number of studies	Pooled sensitivity (95% CI)	I^2* (p value)	Pooled specificity (95% CI)	I^2* (p value)
1 or 2 µg/mL rifampicin					
Direct specimen	6	96·8% (92·4–98·7)	3·3% (0·40)	99·0% (94·3–99·8)	92·3% (<0·0001)
Indirect specimen†	2	100% (95–100)	0% (1·0)	100% (93–100)	0% (1·0)
0·1 µg/mL isoniazid					
Direct specimen	4	96·4% (92·4–98·4)	0% (0·53)	94·2% (83·6–98·1)	89·7% (<0·0001)
Indirect specimen†	2	100% (95–100)	0% (1·0)	98% (89–100)	0% (0·49)
0·4 µg/mL isoniazid					
Direct specimen	7	88·6% (82·7–92·7)	54·1% (0·07)	98·5% (96·8–99·3)	33·0% (0·20)
Indirect specimen†	2	93% (84–98)	13·5% (0·28)	100% (93–100)	0% (1·0)

*I^2 statistic measures the percentage of total variation across studies due to heterogeneity. †Pooled estimates calculated with the univariate random-effects model because too few studies were available for the bivariate random-effects model to converge.

Table 5: **Accuracy of the microscopic-observation drug susceptibility assay when used on direct versus indirect specimens**

0·4 µg/mL cutoff. Subgroup analysis of MODS studies also showed a significant difference between cutoff values of streptomycin (6 µg/mL *vs* 2 µg/mL) for detection of resistance, so results were stratified. For the MODS assay of ethambutol and streptomycin resistance, sensitivity for detection of resistant strains was poor, but specificity for classification of susceptible strains was fairly accurate. In two studies of the MODS assay, a microdilution method was used as the reference standard,[21,22] and a third study used a substantially different protocol to read resistance from the MODS assay.[25] Such study characteristics could have been grounds for exclusion, so we repeated our analysis without these three studies, but none of the estimates changed significantly (table 4).

We did subgroup analyses of two important characteristics of study quality, blinding and sample selection, to explore the effects of these characteristics on pooled estimates of accuracy for detection of rifampicin resistance. Comparison of five studies that clearly reported masked interpretation of the index and use of reference tests with three studies that did not report blinding showed no significant differences in pooled sensitivity or specificity estimates (data not shown). Similarly, differences in pooled estimates of accuracy did not differ between the four studies in which random or consecutive sampling was used to select specimens and the four studies that used convenience sampling or did not clearly report the method of sample selection (data not shown).

Too few studies were available to compare the effects of direct versus indirect inoculation on the performance of the TLA assay. For the MODS assay, sensitivity and specificity for detection of resistance to rifampicin or isoniazid (at either drug concentration) did not differ significantly between the types of inoculate (table 5). However, accuracy estimates with direct specimens were lower in all cases than with indirect specimens (range –3·2% to –4·4% for sensitivity, –1·0% to –3·8% for specificity). No direct head-to-head comparisons were identified for MODS versus TLA drug susceptibility testing on the same specimens, or for direct versus

indirect specimen inoculation.

We plotted sensitivity (or true positives rate) and one minus specificity (or false positives rate) in an HSROC curve for the MODS assay of rifampicin resistance (figure 6) and isoniazid resistance (figure 7). Sensitivity seemed generally more variable than did specificity, and this effect was more pronounced for isoniazid than for rifampicin MODS testing.

Median or mean turnaround times were provided in six MODS studies and two TLA studies, and were stratified by type of inoculate (table 6). Mean turnaround times with the MODS assay were slightly shorter than that with the TLA assay, and, for the MODS assay, use of direct specimens was associated with longer turnaround times than was use of indirect isolates, but 95% CIs overlapped for both comparisons, suggesting that neither difference was significant. All studies reporting bacterial or fungal contamination rates for the MODS or TLA assay used direct specimens for inoculation (table 7). The contamination rate for the TLA assay was significantly lower than that for the MODS assay, with non-overlapping 95% CIs, but data were only available from two TLA studies and three MODS studies.

None of the studies assessing the MODS or TLA assay included a thorough investigation for cross-contamination between cultures. However, in a separate study by Moore and colleagues',[34] a combination of reculturing, molecular typing, and clinical epidemiology was used to identify cross-contaminated cultures from the MODS assay, MB/BacT (Biomerieux, Durham, NC, USA), and Löwenstein-Jensen culture. Of 362 positive samples (11% positivity in 3416 samples), 17 false positive cultures were identified in 14 specimens: 12 with MODS testing, four with MB/BacT, and one with Löwenstein-Jensen culture. Most false positive samples from MODS testing showed evidence of growth in only one drug-free well, and if the definition of a positive culture was revised to demand growth in two drug-free wells, the number of cross-contaminated cultures reduced to three. This amended definition has been incorporated into the standard operating procedure for MODS testing for tuberculosis.[9]

Four studies reported estimates for the cost of drug susceptibility testing with the MODS or TLA assay.[22,23,26,27] Caviedes and colleagues (2000)[22] estimated the reagent and supplies costs for testing against two drugs (rifampicin and isoniazid) to be US$1·72 per sample for the MODS assay and $1·60 per sample for the TLA assay. Equivalent estimates for testing against four drugs (rifampicin, isoniazid, ethambutol, and streptomycin) were $1·80 per sample for MODS testing and $2·92 per sample for TLA testing. Similar costs for MODS testing were subsequently reported in 2006 by the same research group at $2 per sample,[27] and by Mengatto and colleagues (2006)[26] at $1·57 per sample for testing against two drugs and $2·17 per sample for testing against four drugs. Devasia and colleagues (2009)[23] reported costs of $1·38 per sample for MODS testing against ofloxacin (including reagents and supplies) after purchase of an incubator (estimated cost $8000) and an inverted microscope (estimated cost $4000). None of the studies considered the costs of labour, capital costs, or overhead costs associated with MODS or TLA testing. All prices were reported for the year of publication.

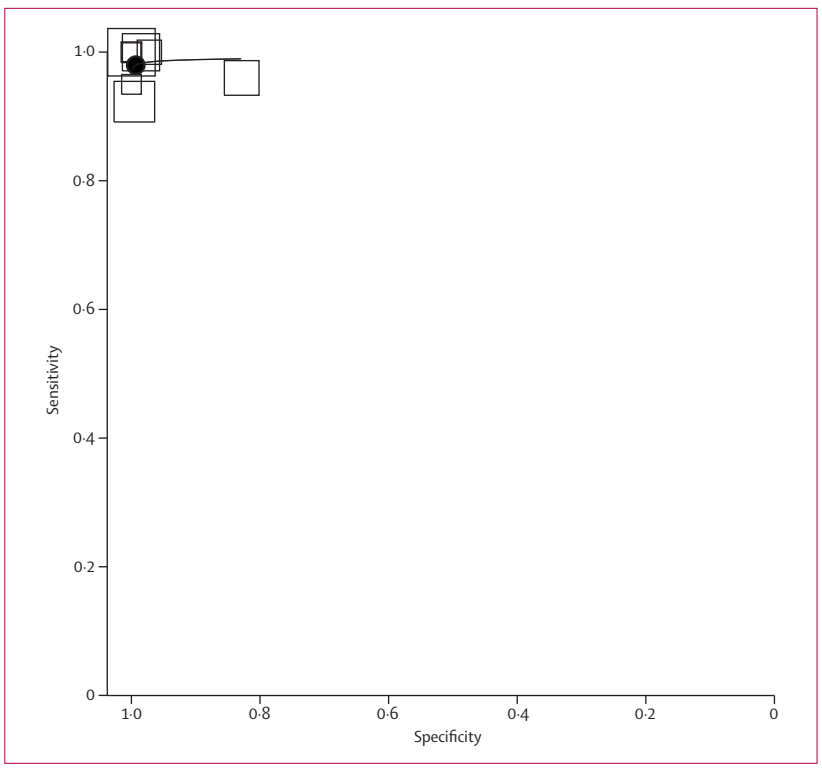

Figure 6: **Hierarchical summary receiver operating characteristic plot of the microscopic-observation drug susceptibility assay for detection of rifampicin resistance**
Open squares represent individual studies, with the size of the square proportionate to the size of the study. The summary point is a closed circle, representing sensitivity and specificity estimates pooled with a bivariate random-effects model. The hierarchical summary receiver operating characteristic curve is truncated outside of the area for which data exist.

Although outcomes important to patients were not directly assessed, Nic Fhogartaigh and colleagues[33] reported a retrospective database analysis of patients from Lima, Peru, whose specimens were selected by their physicians to undergo MODS testing. Of 209 patients with positive culture samples, more than 80% had culture confirmation or availability of the test result from the MODS assay before any standard method. In 41·4% of patients with positive culture samples, the results from MODS testing should have prompted a modification in management of patients.

For more on **MODS testing procedures** see http://www.modsperu.org

Discussion

From pooled estimates of nine studies, MODS testing had high accuracy for detection of rifampicin resistance, but showed slightly lower sensitivity for detection of isoniazid resistance. We identified only three studies assessing the TLA assay; however, in all studies, the assay

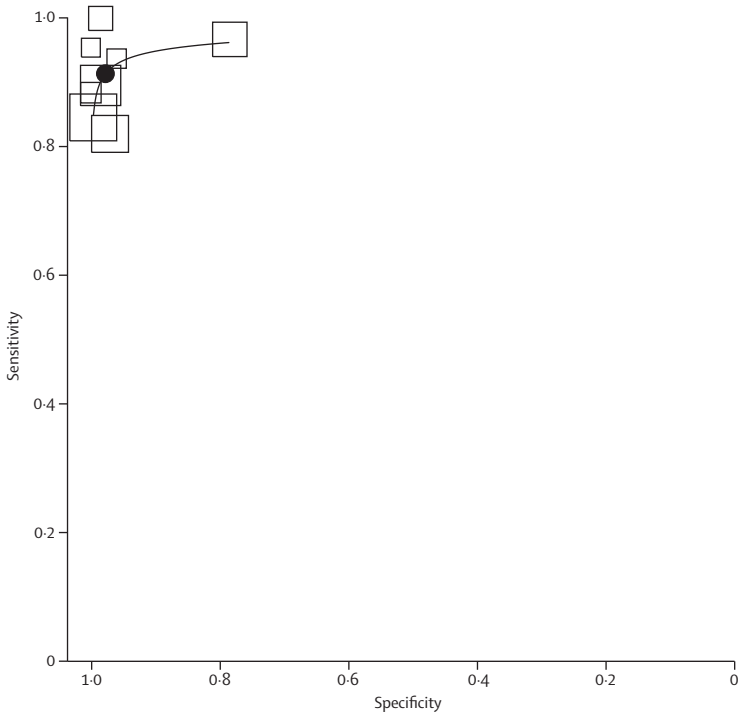

***Figure 7*: Hierarchical summary receiver operating characteristic plot of the microscopic-observation drug susceptibility assay for detection of isoniazid resistance**
Open squares represent individual studies, with the size of the square proportionate to the size of the study. The summary point is a closed circle, representing sensitivity and specificity estimates pooled with a bivariate random-effects model. The hierarchical summary receiver operating characteristic curve is truncated outside of the area for which data exist.

	Microscopic-observation drug susceptibility assay		Thin layer agar assay	
	Number of studies	Time (days)	Number of studies	Time (days)
Overall	6	9·9 (6·0–21·0; 4·1–15·8)	2	11·1 (11·0–11·2; 10·1–12·0)
Direct only	4	11·6 (6·0–21·0; 1·5–21·7)	2	11·1 (11·0–11·2; 10·1–12·0)
Indirect only	2	6·5 (6·0–7·0; 0·2–12·9)	0	..

Data are means of values in original studies (range; 95% CI). Turnaround times are defined as the number of days between specimen receipt or processing in the laboratory to the availability of results within the laboratory.

***Table 6*: Turnaround times**

had 100% concordance with the reference standards for detection of rifampicin or isoniazid resistance.

For detection of isoniazid restistance with the MODS assay, studies in which a cutoff of 0·1 µg/mL was used had significantly higher sensitivity, but somewhat lower specificity, than did studies in which a cutoff of 0·4 µg/mL was used. In view of pharmacokinetic and pharmacodynamic evidence supporting further lowering of isoniazid cutoffs to define resistance, we recommend use of the conservative 0·1 µg/mL cutoff for cases in which only one concentration can be used.[35] MODS testing of ethambutol and streptomycin showed variable specificity but generally low sensitivity. Difficulty with standardisation of susceptibility testing of *M tuberculosis* against many drugs is not unique to the MODS assay and variable concordance between other methods has also been reported.[36] For other antituberculous drugs tested in only one study, the TLA assay had 100% sensitivity and more than 98% specificity.

Although differences in accuracy between studies using direct specimens for inoculation and those using indirect isolates were not significant, direct inoculation was associated with lower sensitivity and specificity. Turnaround times for both the MODS and TLA assays were much faster than is conventional proportion method drug susceptibility testing and similar to commercial liquid systems.[37] For the TLA assay, all turnaround time estimates came from studies using direct specimens, but stratification by specimen type for the MODS assay showed a longer turnaround time for direct inoculation than for indirect inoculation. MODS testing seemed to have a higher contamination rate than did the TLA assay.

This review had several strengths, including a broad and inclusive search of published reports together with efforts to identify unpublished studies. Study selection and data extraction was done independently by two reviewers. Additionally, we used rigorous statistical methods—bivariate random-effects models where possible and HSROC curves for assessment of diagnostic accuracy—which have been recommended by the Cochrane Diagnostic Test Accuracy Working Group as the methods of choice for diagnostic meta-analyses.[12]

However, the review also had several limitations. First, few studies were available for some assessments, especially for the TLA assay and for drugs other than rifampicin and isoniazid, which meant that bivariate models would not converge and some subgroup analyses had to be done with less sophisticated statistical methods. The small number of studies also meant that important study characteristics, including type of reference standard used, method of decontamination, and schedule of microscopic examination, were not assessed. Second, although we specifically reviewed drug susceptibility testing by MODS and TLA and their agreement with reference standards, when these assays are used on direct specimens they are implicitly being used for detection and identification of *M tuberculosis* as well. Our estimates of test accuracy do not account for the variability between studies due to differential isolation or inappropriate speciation from direct specimens; however, a thorough review and meta-analysis of the performance of MODS and TLA assays for diagnosis of active tuberculosis has been done to specifically address this issue.[38] Third, no thorough evaluations of cost-effectiveness were available, and no studies focused specifically on outcomes in patients. The translation of accurate, timely, and useful results into outcomes important to patients is difficult to show and is dependent on many programmatic factors. Nevertheless, Nic Fhogartaigh and colleagues' study[33]

	Contamination rate*	Comparison†	
		Solid culture	Liquid culture
Microscopic-observation drug susceptibility assay	7·4% (0·4–8·1; 6·6–8·2; n=3)	12·9% (1·0–14·2; 11·9–13·9; n=3)	4·2% (4·0–4·4; 3·6–4·9; n=2)
Thin layer agar assay	1·4% (0–4·1; 0·4–3·5; n=2)	10·5% (··; 5·2–18·5; n=1)	2·1%(··; 0·3–7·4; n=1)

Data are means of values in original studies, weighted by the total number of specimens in each study (range; 95% CI; number of studies). *Defined as the proportion of specimens contaminated on first inoculation. †Contamination rate of the reference standard method of isolation done on the same specimens by the same laboratory.

Table 7: **Contamination rates of the microscopic-observation drug susceptibility or thin layer agar assay with comparison to the reference standard method**

provides good evidence that MODS culture could improve care, but this finding needs confirmation in studies with large population samples. Fourth, publication bias is a concern with all systematic reviews. Statistical and graphical approaches for publication bias (eg, funnel plots and regression asymmetry tests) are not recommended for diagnostic meta-analyses.[12] In this context, we are unable to exclude the possibility of bias against publication of studies with poor performance of the MODS or TLA assay.

Microcolony detection methods such as MODS and TLA assays are inexpensive, rapid alternatives for drug susceptibility testing for *M tuberculosis*. Along with the nitrate reductase assay, these non-commercial methods to detect resistance could fulfil a pressing need for tuberculosis diagnostics. Although extensive and stringent studies have been done to prove the accuracy of commercial liquid culture systems and rapid molecular detection of drug resistance both in research settings and under programmatic conditions, such large-scale assessments are unlikely to be funded for non-commercial laboratory techniques such as the MODS or TLA assay. Thus, policy makers and decision makers have to weigh the potential risks and benefits of implementation of diagnostic techniques that are less standardised than are commercial methods and have few data available on their performance in varied real-world settings.

Despite these concerns, the need for rapid, simple, and affordable methods to detect drug-resistant tuberculosis is becoming increasingly urgent as rates of MDR and XDR tuberculosis rise steadily.[39] The goal to strengthen laboratories and build the infrastructure and capacity needed to implement rapid, direct molecular detection and liquid culture technologies is important, but such advances will take time to implement. However, efforts are already underway to scale up the use of rapid line-probe assays in several high-burden countries. Tuberculosis control programmes considering implementation of a non-commercial technique to test drug susceptibility need to carefully assess the capabilities of their laboratories, timelines for anticipated growth, improvements in infrastructure and human resources, and the overall capacity and projected budgets of their programme to establish whether implementation of a non-commercial diagnostic technique, such as the MODS or TLA assay, is feasible, and assess whether a more standardised diagnostic technique, such as commercial liquid culture systems and line-probe assays, could instead be implemented. In addition to line-probe assays that are already approved by WHO, a highly sensitive automated molecular assay (Xpert MTB/RIF, Cepheid, CA, USA) is now an option for detection of both *M tuberculosis* and rifampicin resistance within 2 h with minimal hands-on time.[40] This assay promises to decentralise molecular diagnosis since it can potentially be used at the point of treatment in a microscopy centre or in a tuberculosis or HIV clinic.[41]

Several features of MODS and TLA assays need further research.[42] First, direct inoculation with specimens from patients substantially reduces turnaround time but introduces the greatest risk of variability, threatening the principles of traditional proportion method drug susceptibility testing. Head-to-head studies of direct inoculation with specimens from patients versus indirect inoculation with isolates would help to quantify this variability. Direct inoculation might have to be reserved for smear-positive specimens (similar to molecular detection technologies), but no published data are available to support this recommendation.

Second, MODS and TLA assays both use microcolony detection to identify positive cultures. Qualitative assessment of the specificity of microcolony detection is reported briefly by Caviedes and colleagues;[22] however, rigorous assessment of the ability of technologists to differentiate *M tuberculosis* from non-tuberculous mycobacteria is lacking. The need for an additional species identification test would not only affect the complexity and turnaround time of these diagnostics, but also the biosafety level needed for laboratories using them. If species identification by microcolony detection is indeed highly accurate, sealed cultures prepared for the TLA or MODS assay would not need to be opened after initial inoculation with specimens from patients. Without the need to handle cultured isolates, drug susceptibility testing could be done with low biohazard risk. Until data are available to precisely define the specificity of microcolony cording as definitive identification of *M tuberculosis*, one of the two drug-free wells in the MODS assay could include para-nitrobenzoic acid, a specific inhibitor of *M tuberculosis*, to help distinguish growth of *M tuberculosis* from growth of non-tuberculous mycobacteria (Moore D, London School of Hygiene and Tropical medicine, London, UK, personal communication). Additionally, research is underway in collaboration with the Foundation for Innovative New

Diagnostics to develop a diagnostic test that is similar to the TLA assay but incorporates a simple colorimetric indicator to easily identify growth of *M tuberculosis*.

Third, we have presented cost estimates from the reviewed reports, but these estimates are not a true representation of the costs of non-commercial techniques. The estimates are simple totals of supplies and reagents needed per specimen and therefore are very low, but the additional expenses associated with implementation of the assays could substantially increase costs. Costs associated with labour, equipment, infrastructure, training, and quality control and assurance are likely to differ greatly between microcolony methods and conventional techniques for isolation and drug susceptibility testing, and would vary with the setting of implementation and volume of specimens processed. The increased labour requirements of microcolony methods would make MODS or TLA assays less cost effective in developed countries with high labour costs (JM, unpublished data).

Finally, for research on non-commercial techniques to be comparable across laboratories and settings, common standardised operating procedures should be followed whenever possible. Standardised operating procedures are available from the groups developing MODS and TLA assays and the developers supporting the implementation of the MODS assay have recommended procedures for quality assurance and laboratory accreditation. Researchers wanting to contribute to the development of these techniques are encouraged to refer to these documents and to report on their experiences in diverse and pragmatic settings to build a strong evidence base on which policy makers can base their decisions. Only by use of such globally collaborative strategies will non-commercial techniques gain the level of rigorous evidence provided by profit-driven commercial products.

For **standardised operating procedures for the MODS assay** see http://www.modsperu.org **and the TLA assay** see http://www.tbevidence.org/documents/rescentre/sop/TLA.pdf, and for more on **evidence-based tuberculosis diagnosis** see http://www.tbevidence.org

The data in this report were presented to a WHO expert group in September, 2009, to help guide policy decisions and recommendations on the use of diagnostic tests for rapid detection of drug resistance in *M tuberculosis*. WHO now recommends that selected non-commercial methods for drug susceptibility testing be used as an interim solution in resource-constrained settings, under clearly defined programmatic and operational conditions, in reference laboratories or those with sufficient culture capacity until capacity for genotypic or automated liquid culture drug susceptibility testing is developed.[43] Specifically, WHO endorsed the MODS assay, as a direct or indirect test, for rapid screening of patients with suspected MDR tuberculosis. The WHO expert group agreed that evidence was insufficient to recommend the use of the TLA assay for rapid screening of these patients, but this assay is a promising diagnostic technique and further research is encouraged. WHO's policy emphasises that time to detection of MDR tuberculosis might not be faster with indirect inoculation of these tests than with conventional methods of drug susceptibility testing, and in their present forms these non-commercial assays are unable to detect XDR tuberculosis.[43] These limitations underscore the need for continuing development and evaluation of novel tuberculosis diagnostics.

Contributors

JM developed the study protocol, searched for published reports, selected the studies, did data extraction, analysis, and interpretation, and wrote the report. EL selected the studies and extracted the data, and contributed to data interpretation and writing of the report. DM and MP contributed to development of the study protocol, data interpretation, and writing of the report.

Conflicts of interest

MP was a consultant for the non-profit organisation Foundation for Innovative New Diagnostics at the time that this report was submitted for publication, and has become a consultant for the non-profit organisation the Bill & Melinda Gates Foundation since this report was submitted for publication; neither of these organisations had any involvement in this report. All other authors declare that they have no conflicts of interest.

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