Journal of Antimicrobial Chemotherapy (2007) **59**, 175–183
doi:10.1093/jac/dkl477
Advance Access publication 28 November 2006

JAC

Colorimetric redox-indicator methods for the rapid detection of multidrug resistance in *Mycobacterium tuberculosis*: a systematic review and meta-analysis

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Received 20 August 2006; returned 23 September 2006; revised 27 October 2006; accepted 29 October 2006

***Objectives*: With the spread of multidrug-resistant tuberculosis (MDR-TB) there is increasing demand for new accurate and cost-effective tools for rapid drug susceptibility testing (DST), particularly for developing countries. The reference standard method used today for DST is very slow and cumbersome. Colorimetric assays using redox indicators have been proposed to be used in low-resource countries as rapid alternative culture methods for the detection of resistance especially to rifampicin and isoniazid. These methods appear as promising new tools but their accuracy has not been systematically evaluated.**

***Methods*: We did a meta-analysis to evaluate the accuracy of the colorimetric assays for the detection of rifampicin and isoniazid-resistant tuberculosis among clinical isolates. We searched Medline, PubMed (NCBI), Global health-CAB, EJS-E (EbscoHost), ISI Web, Web of Science and IFCC databases and contacted authors if additional information was needed.**

***Results*: Eighteen studies met our inclusion criteria for rifampicin resistance detection and 16 for isoniazid. We used a summary receiver operating characteristic (SROC) curve to perform meta-analysis and summarize diagnostic accuracy. For both drugs, all studies had a sensitivity and specificity that ranged between 89% and 100%.**

***Conclusions*: There is evidence that colorimetric methods are highly sensitive and specific for the rapid detection of MDR-TB. These new tools could offer affordable technologies for TB laboratories especially in places where resources are limited and where the prevalence of MDR-TB is important and make TB control efforts more effective. Additional studies are needed in high MDR prevalence countries and cost-effectiveness analysis to have more evidence on the utility of these methods. Future developments to detect resistance directly from smear-positive sputum specimens should be taken into consideration to speed up the process.**

Keywords: DST, MDR-TB, Alamar blue, resazurin, MTT

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Introduction

Tuberculosis is a major public health problem, particularly in developing countries and multidrug-resistant strains of *Mycobacterium tuberculosis* (MDR-TB), defined as resistance to at least rifampicin and isoniazid, constitute a serious problem for the efficacy of TB control programmes.[1] With the spread of MDR-TB there is increasing demand for new accurate tools for rapid drug susceptibility testing (DST). Conventional tests for the detection of drug resistance are slow and cumbersome. Laboratory diagnosis is complicated by the fastidious growth requirements of the bacillus. For reference laboratories in high-burden countries, culture is the first step to perform DST. Current conventional methods for DST include the proportion method (PM), the absolute concentration method, the resistance ratio method and the radiometric BACTEC.[2–5] The classical Löwenstein-Jenssen (LJ) or the agar-based medium requires a minimum of 3–6 weeks to produce definitive results.[2–4] The commercial liquid-medium BACTEC 460-TB reduces the turn-around time (TAT) but is expensive and places higher demands on equipment to be routinely used in poor-resource countries.[5,6]

During recent years, a number of studies have evaluated the accuracy of colorimetric methods using different growth indicators for detecting especially rifampicin and isoniazid resistance in *M. tuberculosis* in diverse geographical settings. These methods are faster than the conventional DST method. Colorimetric

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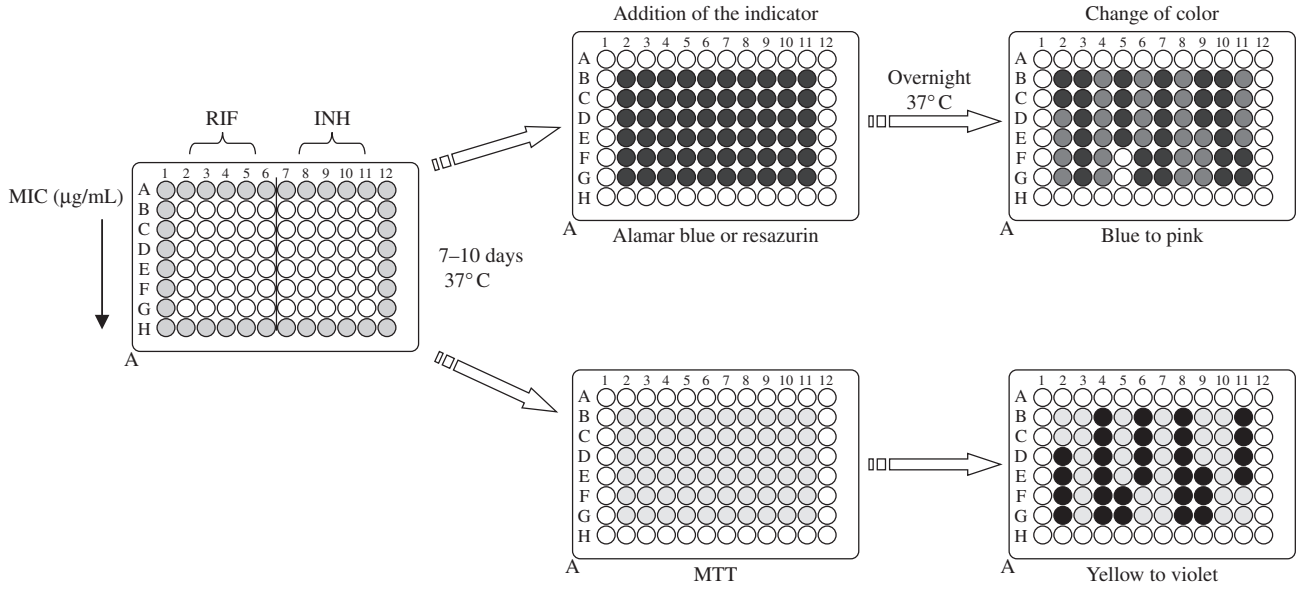

Figure 1. Ninety-six-well microtitre plate for the susceptibility of *M. tuberculosis* isolate using different redox indicators (Alamar blue, resazurin or MTT). MIC is defined as the lowest drug concentration that prevents the change of colour. RIF, rifampicin; INH, isoniazid. A colour version of this figure is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

methods are based on the reduction of a coloured indicator added to the culture medium after *M. tuberculosis* has been exposed *in vitro* to different antibiotics. Resistance is detected by a change in colour of the indicator, which is directly proportional to the number of viable mycobacteria in the medium.[7–10] Different indicators have been evaluated giving comparable results in agreement with the reference standard PM. Among the different growth indicators used are the tetrazolium salts: XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] and MTT [3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide] and the redox indicators Alamar blue and resazurin.

Figure 1 shows a diagram of the microtitre plate assay to perform DST: different concentrations of rifampicin and isoniazid are prepared directly on the plate for MIC determination, and then the bacteria suspension is incubated with the drugs for a few days, followed by the addition of the redox-indicator and visual reading. The most common indicators used to perform the colorimetric assay are: Alamar blue, MTT and resazurin, and no differences in results using these indicators have been observed.

We conducted a systematic review to evaluate the overall accuracy of the colorimetric assays in the detection of rifampicin and isoniazid-resistant TB using a summary receiver operating characteristic (SROC) curve and the area under the curve (AUC) which represents the performance of a diagnostic test based on data from a meta-analysis. The Q* index was used to define the point on the SROC curve where sensitivity and specificity are equal. Systematic reviews of primary studies are becoming important for summarizing evidence about the accuracy of diagnostic tests.[11]

Methods

Search strategy

Data for this review were identified by searches of Medline, PubMed (NCBI), Global health-CAB, EJS-E (EbscoHost), ISI Web, Web of Science and IFCC databases. Search terms (free text, keywords) were '*Mycobacterium tuberculosis*', 'tuberculosis' 'drug susceptibility', 'rifampicin', 'isoniazid' 'colorimetric' 'Alamar blue', 'resazurin', 'MTT', 'redox indicator' for papers published in English from 1966 onwards. All retrieved titles and abstracts were scrutinized for relevant studies about DST of *M. tuberculosis* using the colorimetric methods (MTT, Alamar blue, resazurin or other indicators). In a first step, we did not exclude any study on the basis of a small sample size or not enough data reported.

Study selection

The search through electronic databases returned 28 studies using different kinds of indicator for the rapid detection of rifampicin and isoniazid resistance in *M. tuberculosis*.

We included studies that met the following pre-determined criteria: comparison of the colorimetric assay with a reference standard method[12] (including PM on LJ or Middlebrook agar medium and radiometric BACTEC 460-TB method). Two independent reviewers examined the titles and the abstracts of all identified studies to confirm they had fulfilled the above-defined inclusion criteria. We did not consider studies that did not compare the assay with a reference standard method[13–17] or compared the assay with a method not accepted as reference standard method such as the Alamar blue,[18,19] and viability studies using only the reference strain[20–23] were not included in this review. Two studies[24,25] where the authors used spectrophotometric reading to measure the optical density units (RODU) of the assays instead of a visual reading were also excluded. One study performed the test directly with smear-positive sputum[26] and was not considered in this analysis. The heterogeneity of data was addressed by performing a subgroup analysis with the different redox indicators used. Eighteen reported studies for rifampicin and 16 for isoniazid met eligibility criteria and were included in this review.

Data extraction

Two reviewers independently identified the eligible studies that had fulfilled the above criteria. Data of each article were extracted by one

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Table 1. Description of studies included in the analysis of rifampicin resistance detection

Author, publication year	Country	Indicator used	Number clinical isolates	Reference test	Sample size (no. of resistant/no. of susceptible)	Sensitivity (95% CI)	Specificity (95% CI)	TAT (days)
Yajko *et al.* (1995)[10]	USA	Alamar	50	7H10	9/41	0.89 (0.52–1.00)	0.98 (0.87–1.00)	7–14
Franzblau *et al.* (1998)[31]	USA	Alamar	35	BACTEC	9/26	1.00 (0.66–1.00)	1.00 (0.87–1.00)	8
Palomino *et al.* (1999)[32]	Belgium	Alamar	94	LJ	58/36	1.00 (0.94–1.00)	1.00 (0.90–1.00)	8–10
Foongladda *et al.* (2002)[33]	Thailand	MTT	279	LJ	51/228	0.94 (0.84–0.99)	1.00 (0.98–1.00)	4–7
Palomino *et al.* (2002)[34]	Belgium	resazurin	80	LJ	49/31	1.00 (0.93–1.00)	1.00 (0.89–1.00)	7
Luna *et al.* (2003)[35]	Mexico	Alamar	60	7H11	25/35	0.96 (0.80–0.99)	0.97 (0.85–1.00)	8
Banfi *et al.*(2003)[36]	Italy	resazurin	13	7H11	4/9	1.00 (0.40–1.00)	1.00 (0.66–1.00)	7–14
Lemus *et al.* (2004)[37]	Cuba	resazurin	20	LJ	10/10	1.00 (0.69–1.00)	1.00 (0.69–1.00)	10
Lemus *et al.* (2004)[37]	Cuba	MTT	20	LJ	10/10	1.00 (0.69–1.00)	1.00 (0.69–1.00)	10
Reis *et al.* (2004)[38]	Brazil	Alamar	150	LJ	50/100	1.00 (0.93–1.00)	1.00 (0.96–1.00)	7
Montoro *et al.* (2005)[39]	Cuba	resazurin	100	LJ	37/63	1.00 (0.91–1.00)	0.98 (0.91–1.00)	10
Montoro *et al.* (2005)[39]	Cuba	MTT	100	LJ	37/63	1.00 (0.91–1.00)	1.00 (0.94–1.00)	10
Martin *et al.* (2005)[40]	Belgium	resazurin	203	LJ	102/101	0.98 (0.93–1.00)	0.99 (0.95–1.00)	8
Martin *et al.* (2005)[40]	Belgium	MTT	203	LJ	102/101	0.99 (0.95–1.00)	0.99 (0.95–1.00)	8
Da Silva *et al.* (2006)[41]	Brazil	Alamar	18	LJ	8/10	1.00 (0.63–1.00)	1.00 (0.69–1.00)	7
Da Silva *et al.* (2006)[41]	Brazil	MTT	18	LJ	8/10	1.00 (0.63–1.00)	1.00 (0.69–1.00)	7
Nateche *et al.* (2006)[42]	Algeria	resazurin	136	LJ	12/124	0.92 (0.62–1.00)	0.99 (0.96–1.00)	8
Coban *et al.* (2006)[43]	Turkey	resazurin	50	BACTEC	18/32	1.00 (0.81–1.00)	1.00 (0.89–1.00)	8

Table 2. Description of studies included in the analysis of isoniazid resistance detection

Author, publication year	Country	Indicator used	Number clinical isolates	Reference test	Sample size (no. of resistant/no. of susceptible)	Sensitivity (95% CI)	Specificity (95% CI)	TAT (days)
Yajko *et al.* (1995)[10]	USA	Alamar	50	7H10	29/21	1.00 (0.88–1.00)	0.95 (0.76–0.99)	7–14
Franzblau *et al.* (1998)[31]	USA	Alamar	35	BACTEC	16/19	0.94 (0.70–0.99)	0.89 (0.67–0.99)	8
Palomino *et al.* (1999)[32]	Belgium	Alamar	94	LJ	57/37	1.00 (0.94–1.00)	1.00 (0.91–1.00)	8–10
Foongladda *et al.* (2002)[33]	Thailand	MTT	279	LJ	65/214	0.92 (0.83–0.97)	1.00 (0.97–1.00)	4–7
Palomino *et al.* (2002)[34]	Belgium	resazurin	80	LJ	54/26	1.00 (0.93–1.00)	0.96 (0.80–1.00)	7
Luna *et al.* (2003)[35]	Mexico	Alamar	60	7H11	24/36	0.96 (0.79–1.00)	0.97 (0.85–1.00)	8
Banfi *et al.* (2003)[36]	Italy	resazurin	13	7H11	5/8	1.00 (0.48–1.00)	1.00 (0.63–1.00)	7–14
Reis *et al.* (2004)[38]	Brazil	Alamar	150	LJ	50/100	0.96 (0.86–0.99)	0.99 (0.95–1.00)	7
Montoro *et al.* (2005)[39]	Cuba	resazurin	100	LJ	45/55	1.00 (0.92–1.00)	0.96 (0.87–1.00)	10
Montoro *et al.* (2005)[39]	Cuba	MTT	100	LJ	45/55	1.00 (0.92–1.00)	0.96 (0.87–1.00)	10
Martin *et al.* (2005)[40]	Belgium	resazurin	203	LJ	82/121	0.98 (0.91–1.00)	0.98 (0.93–0.99)	8
Martin *et al.* (2005)[40]	Belgium	MTT	203	LJ	82/121	0.98 (0.91–1.00)	0.98 (0.94–1.00)	8
da Silva *et al.* (2006)[41]	Brazil	Alamar	18	LJ	10/8	1.00 (0.69–1.00)	1.00 (0.63–1.00)	7
da Silva *et al.* (2006)[41]	Brazil	MTT	18	LJ	10/8	1.00 (0.69–1.00)	0.88 (0.47–1.00)	7
Nateche *et al.* (2006)[42]	Algeria	resazurin	136	LJ	17/119	1.00 (0.80–1.00)	0.99 (0.95–1.00)	8
Coban *et al.* (2006)[43]	Turkey	resazurin	50	BACTEC	28/22	0.93 (0.76–0.99)	1.00 (0.85–1.00)	8

reviewer and a sample of these was assessed by a second reviewer to check accuracy in data extraction. We classified data according to the following parameters included in Tables 1 and 2: the indicator used in the assay, the number of isolates tested, the reference standard method used, the sample size, the outcome data (sensitivity and specificity as determined by comparison with the reference standard), the TAT that evaluates the speed of the colorimetric assay which means in how many days results were available.

Data synthesis and meta-analysis

We used standard methods for the diagnostic meta-analysis and performed data analysis using the Meta-DiSc software (version 1.4).[27]

We focused on sensitivity and specificity as measures of diagnostic accuracy of the colorimetric assay. For each article, we created a two by two table of the colorimetric assay rifampicin and isoniazid susceptibility results and cross-tabulated. Sensitivity

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(true positive rate, TPR) was defined as the proportion of isolates determined to be rifampicin or isoniazid resistant by the reference method correctly identified as rifampicin or isoniazid resistant by the colorimetric method. Specificity (true negative rate or false positive rate, FPR) was defined as the proportion of isolates determined to be rifampicin or isoniazid susceptible by the reference method correctly identified as rifampicin or isoniazid susceptible by the colorimetric method.

We created a forest plot to estimate the accuracy of each test. The receiver operating characteristic (ROC) curve is well established as a method of summarizing the performance of a diagnostic test within a single study. It indicates the relationship between the TPR and the FPR of the test. The SROC curve is similar to the ROC curve for a single study except that the data points for the SROC curve are obtained from a set of studies being used for an overview and meta-analysis. The AUC represents an overall summary of the performance of a test. AUC ranges from 1 for a perfect test that always correctly diagnoses, to 0 for a test that never correctly diagnoses. The Q* index represents a summarization of test performance where sensitivity and specificity are equal.[28,29]

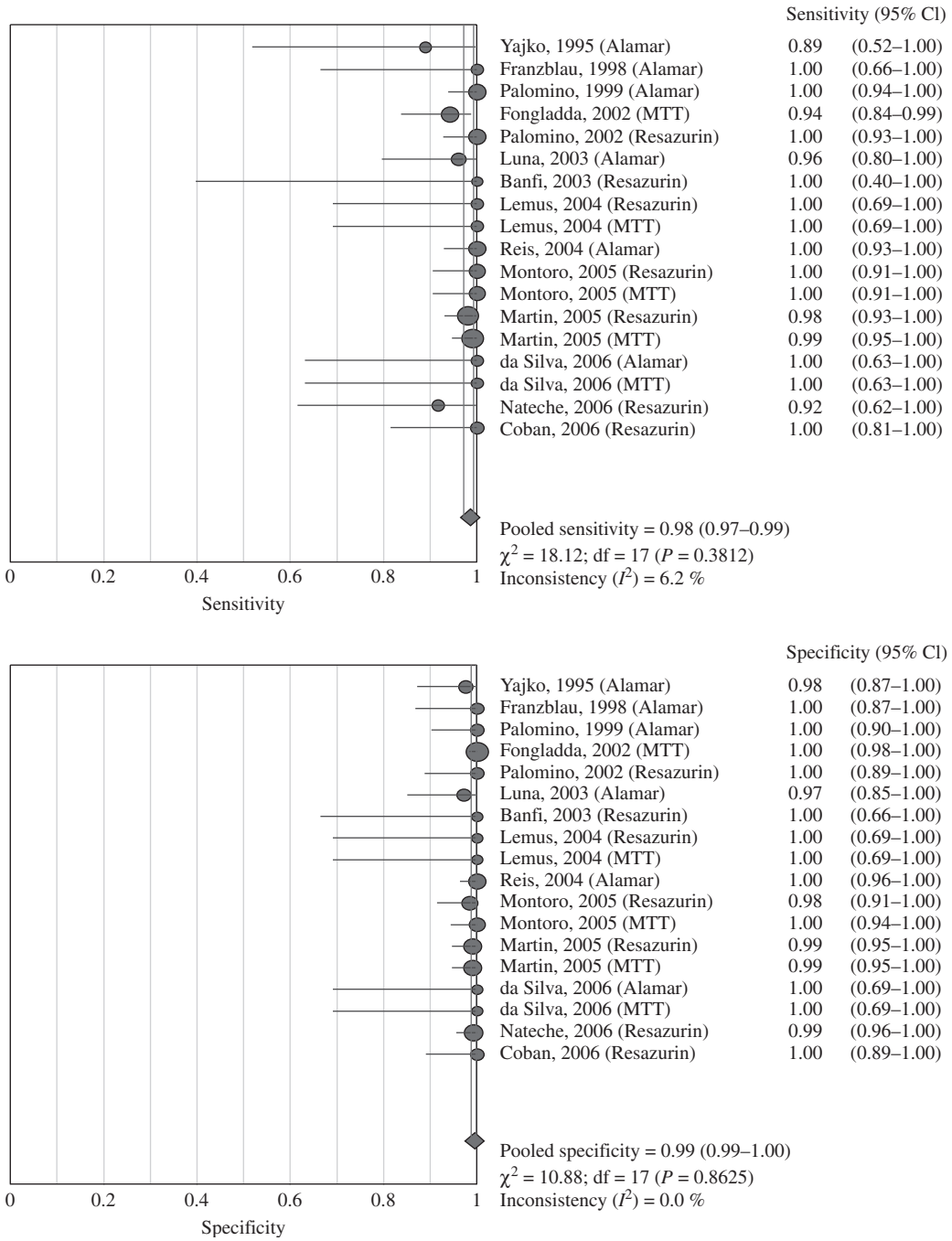

Figure 2. Forest plot of the sensitivity and specificity for rifampicin. The point estimates of sensitivity and specificity from each study are shown as a circles. Error bars are 95% confidence intervals.

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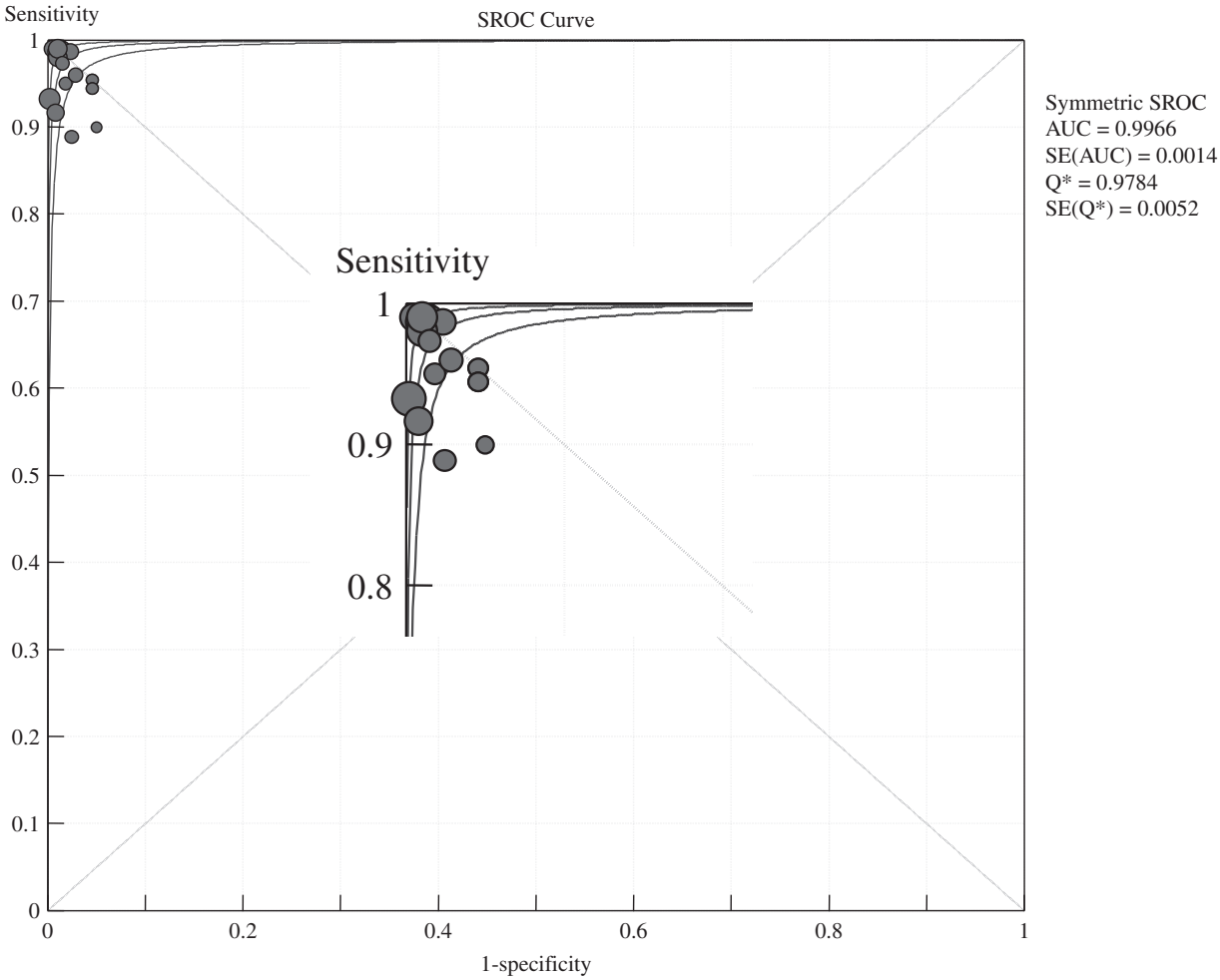

Figure 3. Summary receiver operator curve (SROC) plot for rifampicin colorimetric assay. Each circle represents each study in the analysis. The curve is the regression line that summarizes the overall diagnostic accuracy. SROC, summary receiver operating characteristic; AUC, area under the curve; SE (AUC), standard error AUC; Q*, an index defined by the point on the SROC curve where the sensitivity and specificity are equal, which is the point closest to the top-left corner of the ROC space; SE(Q*), standard error of Q* index.

Quality assessment of included studies

The quality assessment of individual studies was performed by using the QUADAS tool.[30] See Tables S1 and S2 [available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]

Results

Detection of rifampicin resistance

Table 1 describes the characteristics of the 18 included studies for the detection of rifampicin resistance. All studies tested the colorimetric assay on culture isolates. Four studies[37,39–41] are listed twice because the authors tested two different colorimetric indicators and data were extracted and analysed separately. The reference standard method used to compare the assay was either the BACTEC TB-460 or the PM on LJ medium or Middlebrook agar medium. Only two studies[31,43] used the radiometric BACTEC 460-TB system as a reference standard.

Figure 2 illustrates a forest plot that estimates the sensitivity and specificity based on results of the 18 included studies. Figure 3 is a SROC curve of the same data. Of the 18 studies, 11 studies reported sensitivity and specificity of 100%, 4 studies >95%, 2 studies >90% and only 1 study reported sensitivity of 89% and specificity of 98%.

The SROC curve shows an AUC of 0.99 and Q* of 0.97, indicating a high level of overall accuracy.

Detection of isoniazid resistance

Table 2 describes the characteristics of the 16 included studies for the detection of isoniazid resistance. In this case, three studies[39–41] are listed twice because the authors tested two different colorimetric indicators and data were extracted and analysed separately. Figure 4 illustrates a forest plot that estimates the sensitivity and specificity based on results of the 16 included studies. Figure 5 is a SROC curve of the same data. Of the 16 studies, 4 reported sensitivity and specificity of 100%, 8 studies >95%, 2 studies >90%. One additional study reported sensitivity of 99% and specificity of 89% while another, sensitivity of 100% and specificity of 88%.

The SROC curve shows an AUC of 0.99 and Q* of 0.97, indicating a high level of overall accuracy.

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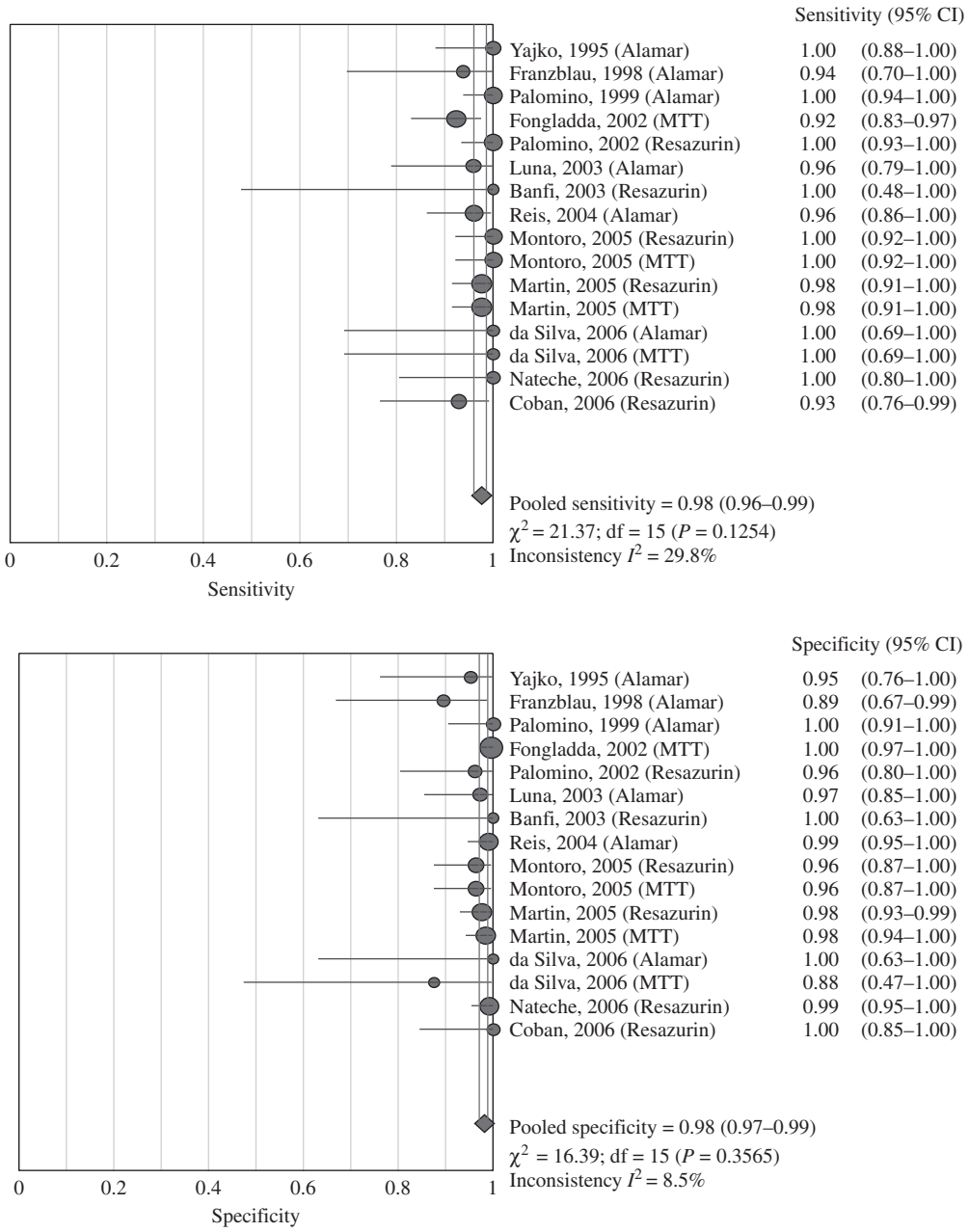

Figure 4. Forest plot of the sensitivity and specificity for isoniazid. The point estimates of sensitivity and specificity from each study are shown as circles. Error bars are 95% confidence intervals.

Colorimetric method performed directly on sputum

We found only one study that tested the colorimetric assay directly on smear-positive clinical specimens for the rapid detection of rifampicin resistance using MTT as indicator. This study showed a sensitivity and specificity of 98.5%.[26]

Discussion

The immediate goal of DST in tuberculosis is the early detection of drug resistance, especially to rifampicin and isoniazid, the two most effective drugs currently available for the treatment of the disease. This allows the early detection of MDR-TB and a better management and treatment of patients. Many developing countries have serious difficulties for obtaining drug susceptibility information on *M. tuberculosis* isolates due to financial or technical constraints. Conventional DST such as the PM on LJ or agar and the BACTEC TB-460 system are time-consuming or need expensive material. This meta-analysis suggests that the colorimetric methods are highly sensitive and specific for the rapid detection of rifampicin and isoniazid resistance in culture isolates. The majority of the studies have a sensitivity of 95%. Although one of the studies[40] provided one-quarter of the pooled results, it did not affect the overall accuracy of the analysis (data not shown).

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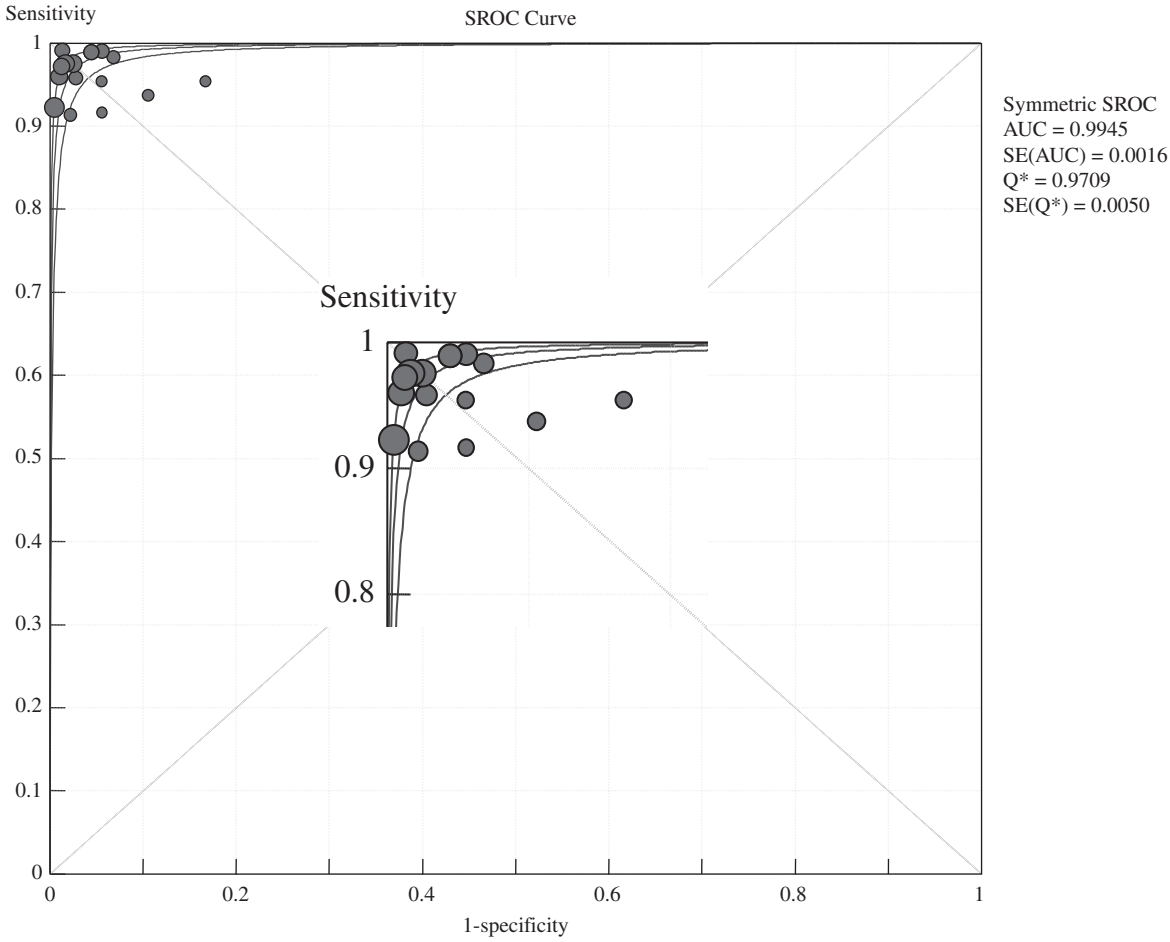

Figure 5. Summary receiver operator curve (SROC) plot for isoniazid colorimetric assay. Each circle represents each study in the analysis. The curve is the regression line that summarizes the overall diagnostic accuracy. SROC, summary receiver operating characteristic; AUC, area under the concentration–time curve; SE (AUC), standard error AUC; Q*, an index defined by the point on the SROC curve where the sensitivity and specificity are equal, which is the point closest to the top-left corner of the ROC space; SE(Q*), standard error of Q* index.

We are confident that our review did not miss any major study recorded in the databases searched. The limitation was the language search since we excluded studies not available in English, which could introduce a bias.

All studies reported the TAT to demonstrate that the colorimetric assays are rapid methods. The average time to have first results was between 7 and 14 days compared with the reference standard method which takes 3–6 weeks. The colorimetric assays are performed on culture isolates, this means that a primary isolation is needed to do the test requiring a minimum of 2–6 extra weeks.

All studies gave similar conclusions in the effort for their implementation in countries with limited resources. These methods can be useful to identify patient populations in which MDR-TB is strongly suspected.

Preliminary calculations indicate that the costs of the colorimetric methods are in the same order of the reference PM.

The overall quality of the included studies was good according to the analysis performed with the QUADAS tool[30] recently described for the assessment of studies of diagnostic accuracy. Additional studies are needed to establish the cost-effectiveness of the colorimetric assays over the conventional method. Studies are also needed to measure the performance of the test in countries with a high prevalence of MDR-TB. Finally, additional research is needed to establish the accuracy of the colorimetric methods applied directly to clinical specimens since only one study is reported in the literature. It will save a great deal of time if tests for MDR-TB can be performed directly on sputum samples.

Acknowledgements

We thank Joris Menten for his advice in statistical analysis. This study was supported in part by the EC LIFESCHIHEALTH-3 (project LSHP-CT-2004-516028) and by the Damien Foundation, Brussels, Belgium.

Transparency declarations

Conflicts of interest: none declared.

Supplementary data

Tables S1 and S2, and a colour version of Figure 1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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References

1. World Health Organization. *Anti-tuberculosis Drug Resistance in the World: Report No. 3. Prevalence and Trends.* The WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance. WHO/HTM/TB/2004.343. Geneva, Switzerland: WHO, 2004.

2. Canetti G, Fox W, Khomenko A *et al*. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. *Bull World Health Organ* 1969; **41**: 21–43.

3. Canetti G, Froman S, Grosset J *et al*. Mycobacteria: laboratory methods for testing drug sensitivity and resistance. *Bull World Health Organ* 1963; **29**: 565–78.

4. Kent PT, Kubica GP. *Public Health Mycobacteriology. A Guide for the Level III Laboratory*. Atlanta: US Department of Health and Human Services, Centers for Disease Control and Prevention, 1985.

5. Heifets LB, Cangelosi GA. Drug susceptibility testing of *Mycobacterium tuberculosis*: a neglected problem at the turn of the century. *Int J Tuberc Lung Dis* 1999; **3**: 564–81.

6. Palomino JC. Nonconventional and new methods in the diagnosis of tuberculosis: feasibility and applicability in the field. *Eur Respir J* 2005; **26**: 339–50.

7. Liu D. A rapid biochemical test for measuring chemical toxicity. *Bull Environ Contam Toxicol* 1981; **26**: 145–9.

8. Tengerdy RP, Nagy JG, Martin B. Quantitative measurement of bacterial growth by the reduction of tetrazolium salts. *Appl Microbiol* 1967; **15**: 954–5.

9. Ali-Vehmas T, Louhi M, Sandholm M. Automation of the resazurin reduction test using fluorometry of microtitration trays. *Zentralbl Veterinarmed B* 1991; **38**: 358–72.

10. Yajko DM, Madej JJ, Lancaster MV *et al*. Colorimetric method for determining MICs of antimicrobial agents for *Mycobacterium tuberculosis*. *J Clin Microbiol* 1995; **33**: 2324–7.

11. Walter SD. Properties of the summary receiver operating characteristic (SROC) curve for diagnostic test data. *Stat Med* 2002; **21**: 1237–56.

12. World Health Organization. *Guidelines for Surveillance of Drug Resistance in Tuberculosis*. (WHO/CDS/CSR/RMD/2003.3). Geneva: WHO, 2003.

13. Kumar M, Khan IA, Verma V *et al*. Rapid, inexpensive MIC determination of *Mycobacterium tuberculosis* isolates by using microplate nitrate reductase assay. *Diagn Microbiol Infect Dis* 2005; **53**: 121–4.

14. Pracharktam R, Angkananukool K, Vibhagool A. *In vitro* susceptibility testing of levofloxacin and ofloxacin by microtiter plate Alamar blue against multidrug and non-multidrug resistant *Mycobacterium tuberculosis* in Thailand. *J Med Assoc Thai* 2001; **84**: 1241–5.

15. Sungkanuparph S, Pracharktam R, Thakkinstian A *et al*. Correlation between susceptibility of *Mycobacterium tuberculosis* by microtiter plate alamar blue assay and clinical outcomes. *J Med Assoc Thai* 2002; **85**: 820–4.

16. Kumar RA, Laiza Paul K, Indulaskshimi R *et al*. Analysis of drug susceptibility in *Mycobacterium tuberculosis* isolated from Thiruvananthapuram using Alamar Blue. *Curr Sci* 2001; **80**: 70–3.

17. Caviedes L, Lee TS, Gilman RH *et al*. Rapid, efficient detection and drug susceptibility testing of *Mycobacterium tuberculosis* in sputum by microscopic observation of broth cultures. The Tuberculosis Working Group in Peru. *J Clin Microbiol* 2000; **38**: 1203–8.

18. Caviedes L, Delgado J, Gilman RH. Tetrazolium microplate assay as a rapid and inexpensive colorimetric method for determination of antibiotic susceptibility of *Mycobacterium tuberculosis*. *J Clin Microbiol* 2002; **40**: 1873–4.

19. Moore DA, Mendoza D, Gilman RH *et al*. Microscopic observation drug susceptibility assay, a rapid, reliable diagnostic test for multidrug-resistant tuberculosis suitable for use in resource-poor settings. *J Clin Microbiol* 2004; **42**: 4432–7.

20. Collins L, Franzblau SG. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob Agents Chemother* 1997; **41**: 1004–9.

21. Mshana RN, Tadesse G, Abate G *et al*. Use of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide for rapid detection of rifampin-resistant *Mycobacterium tuberculosis*. *J Clin Microbiol* 1998; **36**: 1214–19.

22. Abate G, Mshana RN, Miörner H. Evaluation of a colorimetric assay based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) for rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis* 1998; **2**: 1011–16.

23. De Logu A, Uda P, Pellerano ML *et al*. Comparison of two rapid colorimetric methods for determining resistance of *Mycobacterium tuberculosis* to rifampin, isoniazid, and streptomycin in liquid medium. *Eur J Clin Microbiol Infect Dis* 2001; **20**: 33–9.

24. De Logu A, Pellerano ML, Sanna A *et al*. Comparison of the susceptibility testing of clinical isolates of *Mycobacterium tuberculosis* by the XTT colorimetric method and the NCCLS standards method. *Int J Antimicrob Agents* 2003; **21**: 244–50.

25. De Logu A, Borgna R, Uda P *et al*. The 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay as rapid colorimetric method for determination of antibiotic susceptibility of clinical *Mycobacterium tuberculosis* isolates in liquid medium. *Clin Lab* 2003; **49**: 357–65.

26. Abate G, Aseffa A, Selassie A *et al*. Direct colorimetric assay for rapid detection of rifampin-resistant *Mycobacterium tuberculosis*. *J Clin Microbiol* 2004; **42**: 871–3.

27. Zamora J, Abraira V, Muriel A *et al*. Meta-DiSc: a software for meta-analysis of test accuracy data. *BMC Med Res Methodol* 2006; **6**: 31.

28. Walter SD, Macaskill P. *SROC Curve. Encyclopedia of Biopharmaceutical Statistics*. New York: Marcel Dekker, 2004.

29. Walter SD. The partial area under the summary ROC curve. *Stat med* 2005; **24**: 2025–40.

30. Whiting P, Rutjes AW, Reitsma JB *et al*. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol* 2003; **3**: 25.

31. Franzblau SG, Witzig RS, McLaughlin JC *et al*. Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. *J Clin Microbiol* 1998; **36**: 362–6.

32. Palomino JC, Portaels F. Simple procedure for drug susceptibility testing of *Mycobacterium tuberculosis* using a commercial colorimetric assay. *Eur J Clin Microbiol Infect Dis* 1999; **18**: 380–3.

33. Foongladda S, Roengsanthia D, Arjrattanakool W *et al*. Rapid and simple MTT method for rifampicin and isoniazid susceptibility testing of *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis* 2002; **6**: 1118–22.

34. Palomino JC, Martin A, Camacho M *et al*. Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2002; **46**: 2720–2.

35. Luna-Herrera J, Martinez-Cabrera G, Parra-Maldonado R *et al*. Use of receiver operating characteristic curves to assess the performance of a microdilution assay for determination of drug susceptibility of clinical isolates of *Mycobacterium tuberculosis*. *Eur J Clin Microbiol Infect Dis* 2003; **22**: 21–7.

36. Banfi E, Scialino G, Monti-Bragadin C. Development of a microdilution method to evaluate *Mycobacterium tuberculosis* drug susceptibility. *J Antimicrob Chemother* 2003; **52**: 796–800.

37. Lemus D, Martin A, Montoro E *et al*. Rapid alternative methods for detection of rifampicin resistance in *Mycobacterium tuberculosis*. *J Antimicrob Chemother* 2004; **54**: 130–3.

38. Reis RS, Neves I,Jr, Lourenco SL *et al*. Comparison of flow cytometric and Alamar Blue tests with the proportional method for testing susceptibility of *Mycobacterium tuberculosis* to rifampin and isoniazid. *J Clin Microbiol* 2004; **42**: 2247–8.

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39. Montoro E, Lemus D, Echemendia M *et al*. Comparative evaluation of the nitrate reduction assay, the MTT test, and the resazurin microtitre assay for drug susceptibility testing of clinical isolates of *Mycobacterium tuberculosis*. *J Antimicrob Chemother* 2005; **55**: 500–5.

40. Martin A, Morcillo N, Lemus D *et al*. Multicenter evaluation of colorimetric assays using MTT and resazurin for rapid susceptibility testing of *Mycobacterium tuberculosis* to first-line drugs. *Int J Tuberc Lung Dis* 2005; **9**: 901–6.

41. da Silva PA, Boffo M, Mattos I *et al*. Comparison of Redox and D29 phage methods for detection of isoniazid and rifampicin resistance in *Mycobacterium tuberculosis*. *Clin Microbiol Infect* 2005; **12**: 285–8.

42. Nateche F, Martin A, Baraka S *et al*. Application of the resazurin microtitre assay for detection of multidrug resistance in *Mycobacterium tuberculosis* in Algiers. *J Med Microbiol* 2006; **55**: 857–60.

43. Coban AY, Cekic Cihan C, Bilgin K *et al*. Rapid susceptibility test for *Mycobacterium tuberculosis* to isoniazid and rifampin with resazurin method in screw-cap tubes. *J Chemother* 2006; **18**: 140–3.

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