

The nitrate reductase assay for the rapid detection of isoniazid and rifampicin resistance in *Mycobacterium tuberculosis*: a systematic review and meta-analysis

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Objectives: The reference standard methods for drug susceptibility testing (DST) of *M. tuberculosis* are very slow to give results, and due to the emergence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis, there is an urgent demand for new, rapid and accurate DST methods, particularly in low-income countries. The nitrate reductase assay (NRA) has been proposed as a rapid method for the detection of resistance to rifampicin and isoniazid, but its accuracy has not been systematically evaluated.

Methods: We performed a systematic review and meta-analysis to evaluate the accuracy of the NRA for the detection of rifampicin- and isoniazid-resistant tuberculosis. We searched Medline PubMed (NCBI), Global Health-CAB, EJS-E (EbscoHost), ISI Web, Web of Science and IFCC and contacted authors if additional information was required. Fifteen studies met our inclusion criteria for rifampicin resistance detection and 13 for isoniazid. Of these, the majority of the studies used culture isolates on solid medium, four used culture isolates on liquid medium and three used sputum samples. We applied the summary receiver operating characteristic (SROC) curve to perform meta-analysis and to summarize diagnostic accuracy.

Results: For rifampicin, the majority of the studies that applied NRA to isolates had a sensitivity and specificity >94% and for isoniazid, >92%. The three studies that applied NRA directly on sputum samples had a sensitivity and specificity that ranged between 88% and 100%. The SROC curve had an area of >0.99 for both drugs.

Conclusions: There is evidence that NRA is highly sensitive and specific for the rapid detection of rifampicin and isoniazid resistance in culture isolates. More evidence is required for the NRA applied directly on sputum samples, but preliminary results appear promising and show a good sensitivity and specificity. Additional studies are required in countries with a high prevalence of MDR-TB and also cost-effectiveness analysis in order to obtain a complete picture on the utility of this method for rapid drug resistance detection in tuberculosis.

Keywords: drug resistance, *M. tuberculosis*, drug susceptibility testing

Introduction

Early detection of drug resistance in tuberculosis (TB) allows the use of appropriate treatment regimens for the patient, which has an important impact for the better control of the disease.

The development of rapid methods for drug susceptibility testing (DST) is very important due to the increasing rates of multidrug-resistant tuberculosis (MDR-TB) worldwide and the recently described extensively drug-resistant tuberculosis (XDR-TB).^{1,2} The World Health Organization (WHO) and

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members of the STOP TB Partnership called urgently for expanded access to culture and DST in response to the spreading out of MDR-TB and XDR-TB, declared by the WHO as serious emerging threats to public health.³ This poses significant challenges for TB laboratory capacity and the need for faster DST methods. Conventional culture methods using egg- or agar-based media are still the most commonly used approach in many countries. To test for drug resistance, the standard methods using Löwenstein–Jensen (LJ) medium include the proportion method, the absolute concentration method and the resistant ratio method, which are well standardized with clinical isolates, at least for the major antituberculosis drugs. The proportion method was developed in the 1960s and is still ‘the gold standard method’ used in many laboratories, especially in developing countries, because it is an inexpensive method easily accessible in these settings.^{4,5} During recent years, due to the long turnaround time of conventional DST methods, several new approaches have been proposed for the faster detection of MDR-TB, including both genotypic and phenotypic methods.^{6,7} Among the phenotypic methods proposed, the nitrate reductase assay (NRA) is a simple technique based on the capacity of *M. tuberculosis* to reduce nitrate to nitrite, which is detected by adding the Griess reagent to the medium. In 1879, Griess,⁸ a German chemist working at the University of Marburg, described the diazotization reaction, which would form the basis for the Griess test for the detection of nitrite. By incorporating 1 mg/mL potassium nitrate (KNO₃) in the LJ medium, the reduction of nitrate can be detected using the Griess reagent, which produces a coloured reaction. In the presence of rifampicin or isoniazid at the critical concentration, the appearance of a red–pink colour represents resistance to the drug. Susceptible strains will lose the capacity to reduce nitrate, thus producing a non-coloured reaction, as they are inhibited by the antibiotic. Results can be obtained faster than by visual detection of colonies, as the NRA uses the detection of nitrate reduction as an indicator of growth. Progress has been made in the use of the NRA for DST in *M. tuberculosis* in the last 5 years. However, it is essential to further evaluate this new procedure before bringing it into routine laboratory diagnosis. We conducted a systematic review and meta-analysis to synthesize all available literatures on the NRA for DST in *M. tuberculosis* and to evaluate the overall accuracy of this method for the detection of MDR-TB in isolates and in sputum samples.

Methods

Literature search

The most commonly employed search strategy used the Medline PubMed (NCBI) database. Additional databases such as Global Health-CAB, EJS-E (EbscoHost), ISI Web, Web of Science and IFCC were also included. Search terms (free text and keywords) were ‘nitrate reductase’, ‘griess’, ‘nitrate test’, ‘nitratase’, ‘*Mycobacterium tuberculosis*’, ‘tuberculosis’, ‘drug susceptibility’, ‘drug resistance’, ‘multidrug resistance’, ‘diagnosis’, ‘rifampicin’, ‘rifampin’ and ‘isoniazid’ for papers published in English from 1966 onwards. All retrieved titles and abstracts were scrutinized for relevant studies on drug resistance detection of *M. tuberculosis* using the NRA.

Study selection

The search through the electronic databases returned studies using the NRA for rapid DST in *M. tuberculosis*. We identified results from all primary studies evaluating the accuracy (sensitivity and specificity) of

the NRA for the rapid detection of rifampicin- and isoniazid-resistant tuberculosis in *M. tuberculosis* isolates or in sputum samples. We included studies that met the following predetermined criteria: comparison of the NRA with a reference standard method (including proportion method, absolute concentration method, resistance ratio method or radiometric BACTEC 460-TB method); detection of rifampicin and isoniazid resistance; studies that reported data on false-positive, true-positive, false-negative and true-negative results. Our initial search had no language restrictions, but studies not available in the English language were excluded from the data extraction process. The heterogeneity of data was addressed by performing a subgroup analysis with the NRA performed on isolates, in solid or in liquid medium, and on sputum samples.

Data extraction

Two independent reviewers examined the titles and abstracts of all identified studies to confirm that they had fulfilled the above-defined inclusion criteria. Titles and abstracts were first read independently by the two reviewers, and then all papers considered possibly eligible were reviewed independently by the authors who assessed whether the paper was concerned with the NRA for DST. The bibliographies of selected articles were screened for potentially suitable references, which were then retrieved. Those studies that did not match with our requirements were taken out. Data from each article were extracted by one reviewer and a sample of these was assessed by a second reviewer to check the accuracy of data extraction. Articles were examined in detail, and any disagreement was resolved by consensus with a third author. We classified data according to the following parameters included in Tables 1 and 2: the reference standard method used, type of sample (isolates or sputum), the sample size, the outcome data (sensitivity and specificity as determined by comparison with the reference standard) and the time to positivity (TTP) that evaluates the speed of the NRA, which means in how many days results were available.

For each study included, data were also extracted to generate a two-by-two table to estimate the sensitivity and the specificity of the NRA. All extracted data were double-checked by a second author. Sensitivity [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 NRA 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 NRA method.

Data synthesis and meta-analysis

We performed the meta-analysis in accordance with published guidelines^{9,10} and performed the data analysis using the Meta-DiSc software (version 1.4).¹¹

We created a forest plot to estimate the accuracy of each test and the receiver operating characteristic (ROC) curves that are well established as methods for 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 summary ROC (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. The 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 the test performance where sensitivity and

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Table 1. Description of studies included in meta-analysis for rifampicin resistance detection (15 studies)

References	Countries	Reference test	Samples	Sample size (no. resistant/ no. susceptible)	Sensitivity (95% CI)	Specificity (95% CI)	TTP
Angeby <i>et al.</i> ¹⁶	Sweden	BACTEC 460	isolates	31/26	1.00 (0.89–1.00)	1.00 (0.87–1.00)	7
Syre <i>et al.</i> ¹⁷	Norway	BACTEC 460	isolates and liquid	16/57	0.94 (0.71–1.00)	1.00 (0.94–1.00)	5
Lemus <i>et al.</i> ¹⁸	Cuba	PM	isolates	10/10	1.00 (0.69–1.00)	1.00 (0.69–1.00)	10
Coban <i>et al.</i> ¹⁹	Turkey	PM	isolates	8/72	1.00 (0.63–1.00)	1.00 (0.95–1.00)	12
Sethi <i>et al.</i> ²⁰	India	PM	isolates	35/65	1.00 (0.90–1.00)	1.00 (0.94–1.00)	10
Montoro <i>et al.</i> ²¹	Cuba	PM	isolates	37/63	1.00 (0.91–1.00)	1.00 (0.94–1.00)	10
Martin <i>et al.</i> ²²	Belgium/Cuba/ Argentina/Chile	PM	isolates	75/75	0.96 (0.89–0.99)	1.00 (0.95–1.00)	10
Musa <i>et al.</i> ²⁸	Argentina	PM	sputum	11/110	1.00 (0.72–1.00)	1.00 (0.97–1.00)	14
Solis <i>et al.</i> ²⁹	Peru	PM	sputum	101/84	0.94 (0.87–0.97)	1.00 (0.96–1.00)	21
Kumar <i>et al.</i> ²³	India	PM	isolates and liquid	10/43	1.00 (0.69–1.00)	1.00 (0.92–1.00)	8
Kumar <i>et al.</i> ²⁴	India	PM	isolates and liquid	10/32	1.00 (0.69–1.00)	1.00 (0.89–1.00)	7
Poojary <i>et al.</i> ²⁵	India	PM	isolates and liquid	25/74	0.96 (0.80–1.00)	1.00 (0.95–1.00)	6.3
Mengatto <i>et al.</i> ²⁶	Argentina	PM	isolates	25/39	1.00 (0.86–1.00)	1.00 (0.91–1.00)	10
Lemus <i>et al.</i> ²⁷	Cuba	PM	isolates	46/271	0.94 (0.83–0.99)	1.00 (0.99–1.00)	10
Affolabi <i>et al.</i> ³⁰	Benin	PM	sputum	7/169	0.88 (0.47–1.00)	1.00 (0.98–1.00)	18

PM, proportion method.

Table 2. Description of studies included in the meta-analysis for isoniazid resistance detection (13 studies)

References	Countries	Reference test	Samples	Sample size (no. resistant/ no. susceptible)	Sensitivity (95% CI)	Specificity (95% CI)	TTP
Angeby <i>et al.</i> ¹⁶	Sweden	BACTEC 460	isolates	32/23	0.97 (0.84–1.00)	0.96 (0.79–1.00)	7
Syre <i>et al.</i> ¹⁷	Norway	BACTEC 460	isolates and liquid	32/40	1.00 (0.89–1.00)	0.95 (0.84–0.99)	5
Coban <i>et al.</i> ¹⁹	Turkey	PM	isolates	13/67	1.00 (0.75–1.00)	1.00 (0.95–1.00)	12
Sethi <i>et al.</i> ²⁰	India	PM	isolates	32/67	0.97 (0.84–1.00)	1.00 (0.95–1.00)	10
Montoro <i>et al.</i> ²¹	Cuba	PM	isolates	43/55	0.96 (0.85–0.99)	1.00 (0.94–1.00)	10
Martin <i>et al.</i> ²²	Belgium/Cuba/ Argentina/Chile	PM	isolates	58/87	0.97 (0.88–1.00)	0.97 (0.91–0.99)	10
Musa <i>et al.</i> ²⁸	Argentina	PM	sputum	13/107	0.93 (0.66–1.00)	1.00 (0.97–1.00)	14
Solis <i>et al.</i> ²⁹	Peru	PM	sputum	113/78	0.99 (0.95–1.00)	1.00 (0.95–1.00)	21
Kumar <i>et al.</i> ²³	India	PM	isolates and liquid	14/39	1.00 (0.77–1.00)	1.00 (0.91–1.00)	8
Kumar <i>et al.</i> ²⁴	India	PM	isolates and liquid	13/27	0.87 (0.60–0.98)	1.00 (0.87–1.00)	7
Poojary <i>et al.</i> ²⁵	India	PM	isolates and liquid	30/67	0.94 (0.79–0.99)	0.99 (0.92–1.00)	6.3
Mengatto <i>et al.</i> ²⁶	Argentina	PM	isolates	24/38	0.92 (0.75–0.99)	1.00 (0.91–1.00)	10
Lemus <i>et al.</i> ²⁷	Cuba	PM	isolates	55/260	0.92 (0.82–0.97)	1.00 (0.99–1.00)	10

PM, proportion method.

specificity are equal. The heterogeneity among studies was analysed using the heterogeneity χ^2 and I^2 index (interpreted as the percentage of the total variability in a set of effect sizes due to true heterogeneity) included in the Meta-DiSc program.¹² The potential

presence of publication bias was assessed with a funnel plot and the Egger test.^{13,14} These analyses were performed by using the commands for the meta-analysis of diagnostic studies in STATA software (version 8.0; Stata Corporation, College Station, TX, USA).

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Quality assessment

We assessed the study quality of individual studies using the criteria based on the QUADAS tools for the assessment of quality of diagnostic studies;¹⁵ see Tables S1 and S2 [available as Supplementary data at *JAC* Online (<http://jac.oxfordjournals.org/>)].

Results

Detection of rifampicin resistance

Fifteen studies reporting results of rifampicin resistance detection met eligibility criteria and are included in this review. Table 1 describes the characteristics and outcomes of these studies.

Twelve studies performed the NRA on culture isolates and three on sputum samples. From the 12 that performed the NRA on isolates, 4 used a liquid medium format. These studies are recorded in Table 1, in order to describe the outcome of the subgroup analysis. All studies were published between 2002 and 2007. Figure 1 illustrates the forest plots that estimate the sensitivity and specificity based on the results of all the included studies. For the 15 studies, sensitivity has been reported between 88% and 100%, and a pooled sensitivity of 97% was found (Figure 1) and the specificity was exceptionally reported as 100% for all studies. Figure 2 shows the SROC curve for the same data and shows an AUC of 0.9972 and a Q^* of 0.98, indicating a high level of overall accuracy. There was no significant heterogeneity ($\chi^2 = 2.58$ with $df = 14$; $P = 1.0$), and the I^2 index was 0.0%.

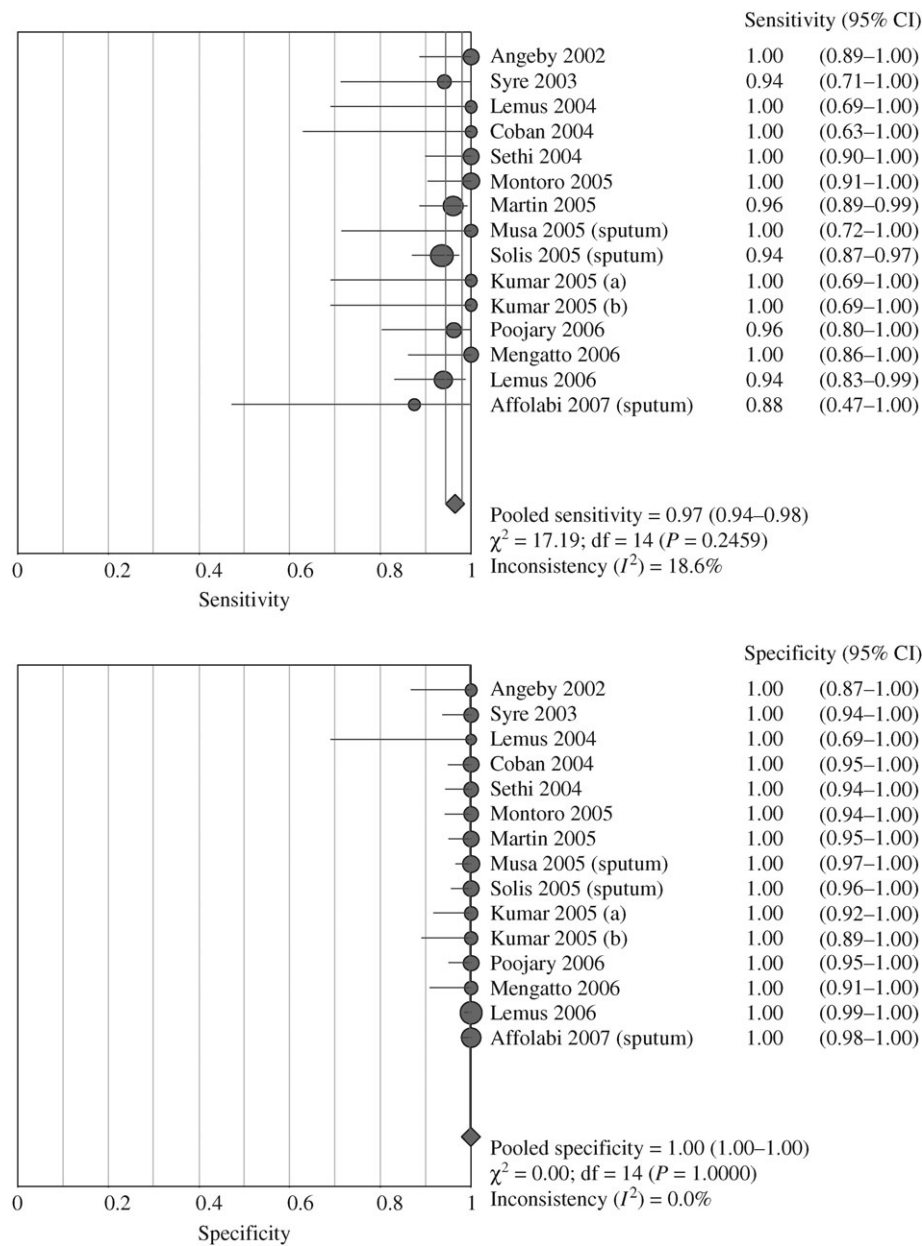


Figure 1. Forest plots of the sensitivity and specificity for rifampicin. The point estimates of sensitivity and specificity from each study are shown as circles. Error bars are 95% confidence intervals.

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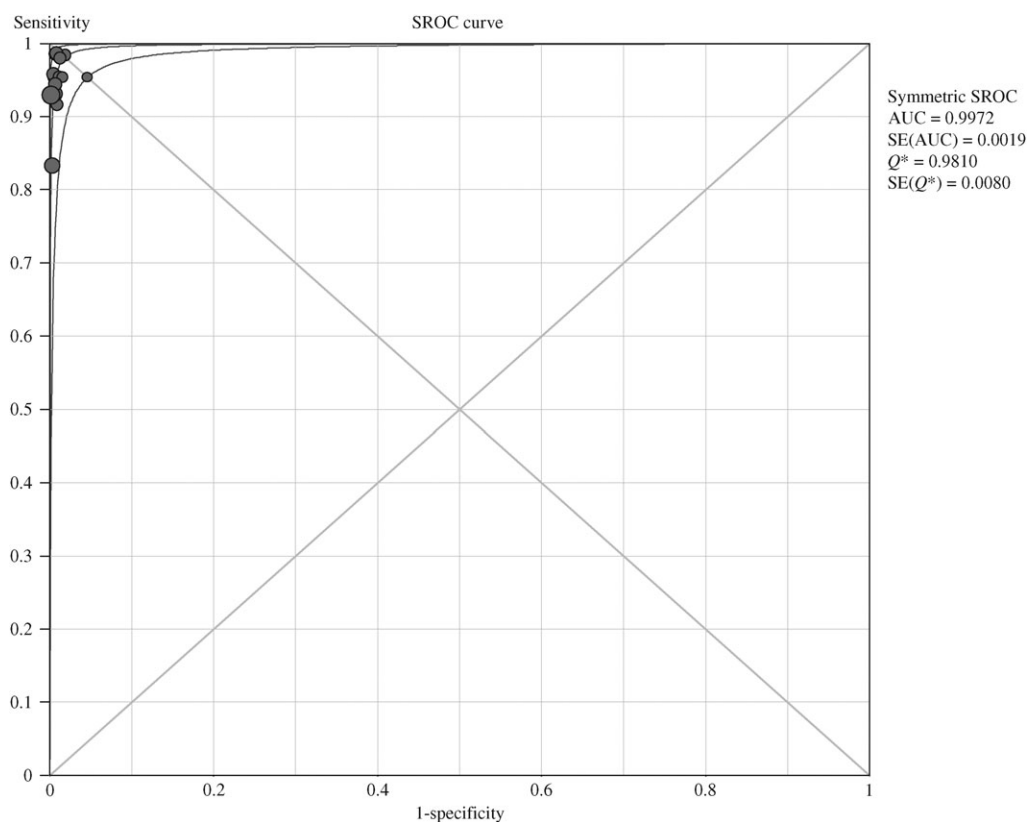


Figure 2. SROC curve for rifampicin. Each circle represents each study in the analysis. The curve is the regression line that summarizes the overall diagnostic accuracy. SROC, summary receiver operating characteristic; AUC, area under the concentration–time curve; SE (AUC), standard error AUC; Q^* , 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.

Subgroup analysis of accuracy of NRA on isolates—solid and liquid media. We performed a subgroup analysis of the studies that applied the test on isolates or on sputum samples separately. As it can be seen in Figure 1, the 12 studies that performed the NRA on isolates had a sensitivity between 94% and 100% and a specificity of 100%.^{16–27} Studies that used a liquid medium format^{17,23–25} had an average TTP of 6.5 days compared with 7–12 days for those that performed the NRA in the solid medium (Table 1).

Subgroup analysis of accuracy of NRA on sputum samples. As illustrated in Figure 1, the three studies that applied the NRA directly on sputum samples^{28–30} reported a sensitivity that ranged between 88% and 100%. The lowest sensitivity, however, was reported in a study with only seven true resistant isolates, out of which one was missed with the NRA.³⁰ The specificity was still high at 100% and the TTP ranged from 14 to 21 days.

Detection of isoniazid resistance

Table 2 describes the characteristics of the 13 studies that reported results for isoniazid resistance detection. Only two studies performed the NRA directly on sputum samples.^{28,29} Figure 3 shows the forest plots that estimate the sensitivity and specificity based on results of all the studies. The sensitivity was between 87% and 100%, and the specificity was higher, between 95% and 100%. Figure 4 shows the SROC curve, indicating an

AUC of 0.9946 and a Q^* of 97, indicating a high level of overall accuracy. There was no significant heterogeneity ($\chi^2 = 4.78$ with $df = 12$; $P = 0.965$), and the I^2 index was 0.0%.

Subgroup analysis of accuracy of NRA on isolates—solid and liquid media. Ten studies that performed the NRA on isolates had a sensitivity that ranged between 92% and 100%.^{16,17,19–23,25–27} One study had a lower sensitivity of 87%.²⁴ The specificity ranged between 95% and 100% (Figure 3). The TTP of these studies was between 5 and 12 days (Table 2). As for the detection of rifampicin resistance, studies that used a liquid medium format^{17,23–25} had an average TTP of 6.5 days compared with 7–12 days for those that performed the NRA in the solid medium (Table 2).

Subgroup analysis of accuracy of NRA on sputum samples. As illustrated in Figure 3, only two studies^{28,29} tested NRA directly on sputum samples to detect the resistance to isoniazid and the sensitivity was reported as 93% and 99%. Specificity was 100% in both studies. The TTP ranged between 14 and 21 days (Table 2).

Publication bias

The Egger test for publication bias was not statistically significant ($P = 0.87$) for the data from studies for the detection of rifampicin resistance, and the funnel plot did not show asymmetry (Figure 5). However, for the detection of isoniazid resistance, the Egger test was significant ($P = 0.02$), with

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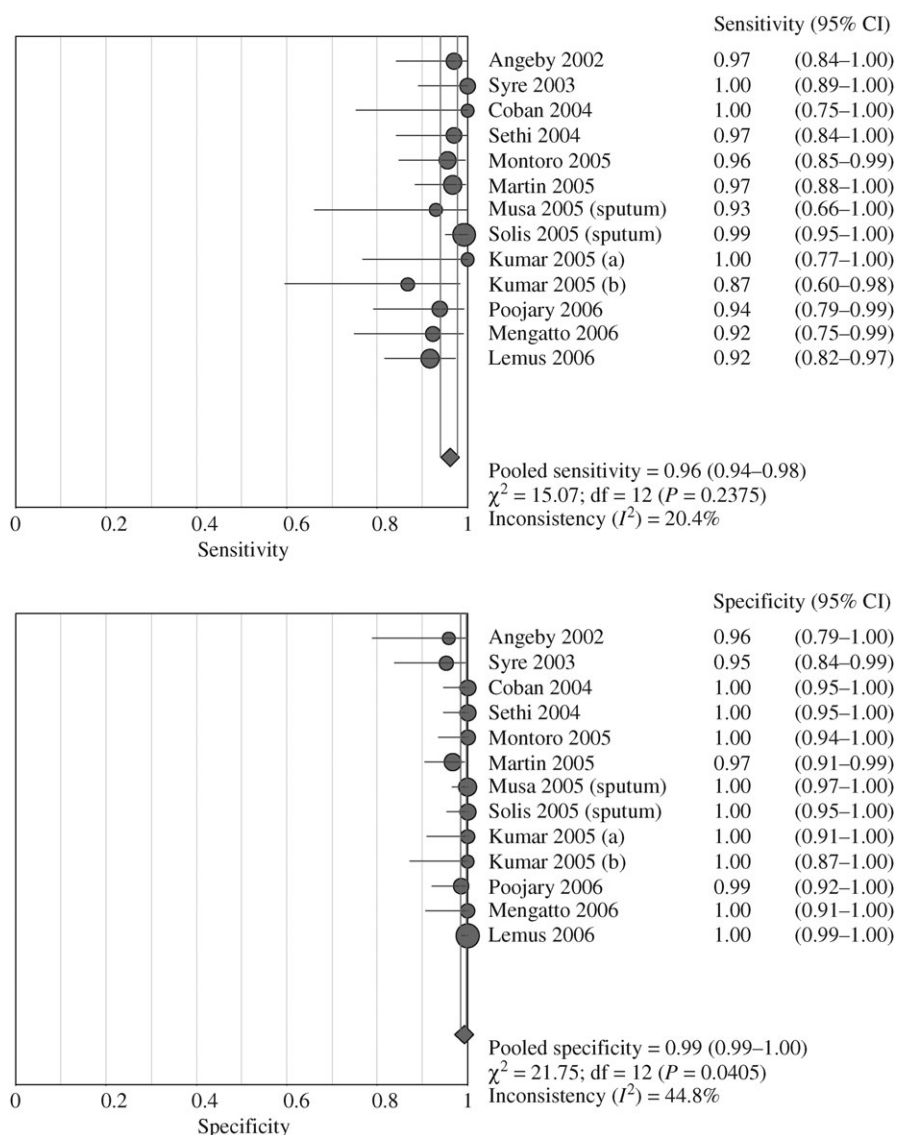


Figure 3. Forest plots of the sensitivity and specificity for isoniazid. The point estimates of sensitivity and specificity from each study are shown as circles. Error bars are 95% confidence intervals.

an asymmetric funnel plot (Figure 6), indicating potential publication bias.

Discussion

The goal of DST is the early detection of drug resistance, especially to rifampicin and isoniazid, the two most effective drugs currently available for the treatment of TB. This allows the early detection of MDR-TB and a better management and treatment of patients. The early identification of MDR-TB cases would decrease the risk of disease and possible amplification of drug resistance. The methods currently available for rapid DST of *M. tuberculosis* are cheap but slow, or fast but too costly to be applicable in most high incidence TB areas. There is obviously a great need for fast, reliable and inexpensive methods for DST of *M. tuberculosis*. However, any new rapid DST method must be carefully calibrated with representative isolates of *M. tuberculosis*

in order to determine *in vitro* the cut-off for resistant and susceptible isolates with acceptable reproducibility.

The present meta-analysis suggests that the NRA is highly sensitive and specific for detecting rifampicin- and isoniazid-resistant TB both in culture isolates and directly on sputum samples. The majority of the studies had a sensitivity of 95% or greater, and nearly all were 100% specific. Even in the subgroup analysis, all studies yielded consistently high estimates of sensitivity and specificity, so heterogeneity was not detected in this meta-analysis. The NRA has shown a high degree of accuracy when used on culture isolates, but this requires 2–6 weeks for primary isolation of the bacteria. Only three studies have applied the NRA directly on sputum samples for rifampicin resistance detection and just two for isoniazid resistance. Additional studies are required to better establish the accuracy of NRA applied to sputum, but preliminary studies suggest that NRA may be useful for rifampicin and isoniazid resistance detection in sputum samples. The accuracy was, in general, slightly higher for rifampicin resistance detection;

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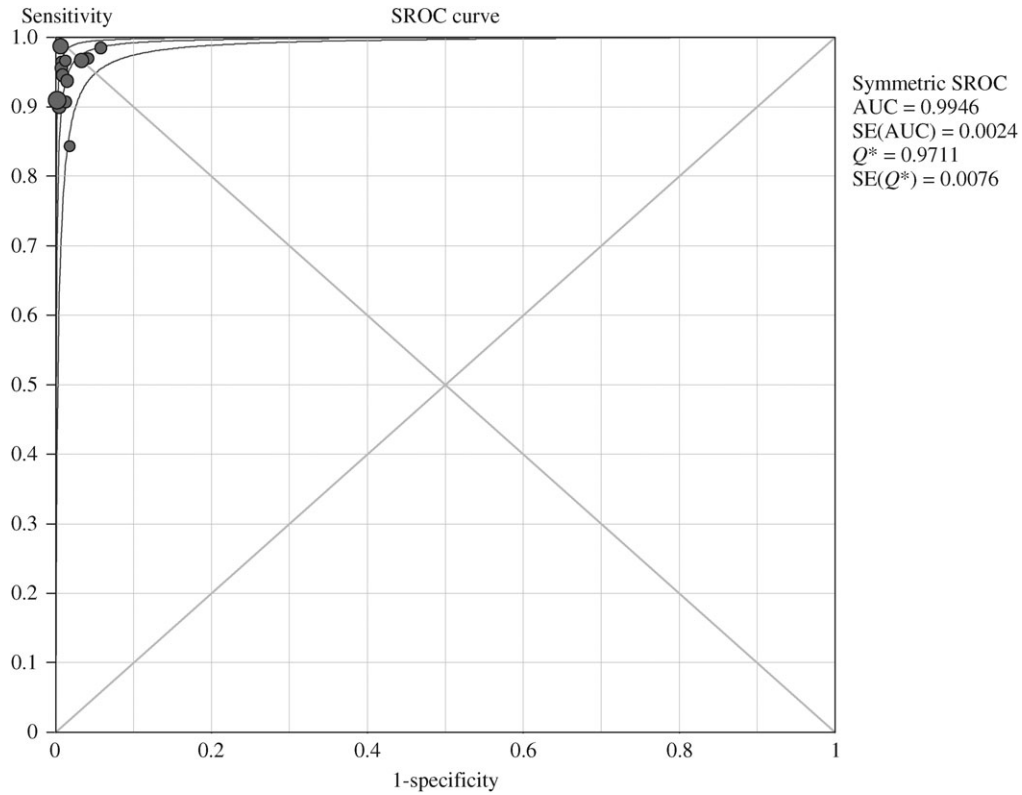


Figure 4. SROC curve for isoniazid. Each circle represents each study in the analysis. The curve is the regression line that summarizes the overall diagnostic accuracy. SROC, summary receiver operating characteristic; AUC, area under the concentration–time curve; SE (AUC), standard error AUC; Q^* , an 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.

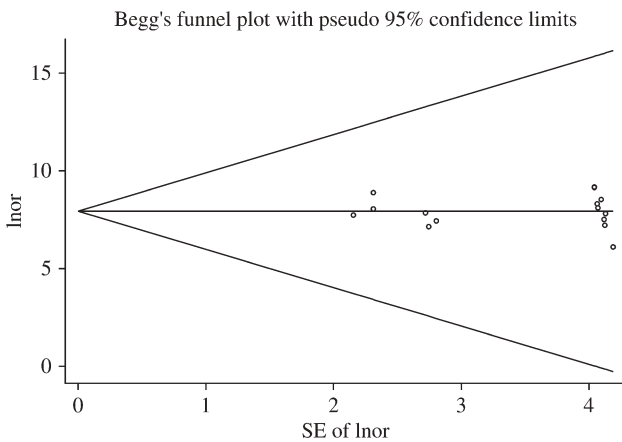


Figure 5. Funnel plot for the evaluation of publication bias for rifampicin. The funnel graph plots the log odds ratio (DOR) versus the standard error of the log of the DOR (an indicator of sample size). Each open circle represents each study in the meta-analysis. The line in the centre indicates the summary DOR. In the absence of publication bias, the DOR estimates from smaller studies are expected to be scattered above and below the summary estimate, producing a triangular or funnel shape.

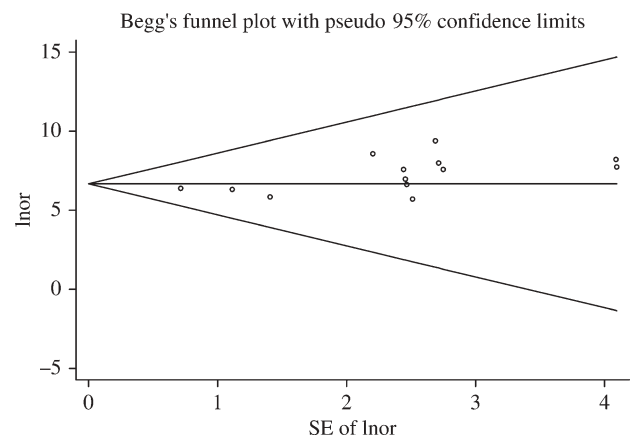


Figure 6. Funnel plot for the evaluation of publication bias for isoniazid. The funnel graph plots the log odds ratio (DOR) versus the standard error of the log of the DOR (an indicator of sample size). Each open circle represents each study in the meta-analysis. The line in the centre indicates the summary DOR. In the absence of publication bias, the DOR estimates from smaller studies are expected to be scattered above and below the summary estimate, producing a triangular or funnel shape.

however, isoniazid resistance detection results also showed high sensitivity and specificity even when applied in sputum samples.

All studies have reported results showing that the NRA is a faster method compared with the conventional method. The

average TTP was between 5 and 12 days when performed on isolates and between 14 and 21 days when used directly on sputum samples, compared with the reference standard method that takes 4–6 weeks when performed on LJ medium or

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3 weeks when performed on agar with 2–3 additional weeks for the first isolation.

The biggest advantage of the NRA is that it is performed in the classical LJ medium that TB laboratories use routinely for the diagnosis of TB. This is a very important factor because laboratories do not have to change completely to another new method, as adaptation to a novel technology is not always easy to implement in laboratories involved in routine work. No equipment is required to perform the NRA, giving the opportunity for its widespread application. Results are simple to interpret by a change of colour. Also, biosafety problems are limited as the test is performed in a solid medium reducing the risk of production of aerosols during manipulation. A limitation of the NRA is that this method cannot be used for nitrate reductase negative *M. tuberculosis* samples, but nitrate reductase negative strains of *M. tuberculosis* are unusual. DST would not be possible to be performed for *Mycobacterium bovis*.

We are confident that our analysis did not miss any major study recorded in the databases searched. One weakness of this review might be that we excluded studies not available in languages other than English. Publication bias was a concern with the detection of isoniazid resistance, and exclusion of studies published in languages other than English could have contributed to this potential bias. In 2002, Panaiotov and Kantardjiev,³¹ in Bulgaria, described the NRA for DST of *M. tuberculosis* strains. The results obtained were in accordance with the results of the proportion method and were obtained within 8–10 days. The technique was in use since 1980 by Emil Kalfin from the National Center of Lung Disease in Bulgaria. Between 1998 and 2000, the same team modified and improved the NRA with the use of a crystalline nitrate reductase reagent. This crystalline reagent is reported to be less toxic and to have a longer shelf-life than the Griess reagent. In 2003, Golyshevskaja *et al.*,³² in an article published in Russian, compared the NRA with the BACTEC 960 system for the detection of resistance to first-line drugs. In 2006, Poliakov *et al.*,³³ in Russia, performed NRA directly on sputum samples for the detection of rifampicin and isoniazid resistance. After reviewing the abstracts of these papers, it suggests that the overall results are similar to the results of the studies included in this analysis.

The overall quality of the included studies was good according to the analysis performed with the QUADAS tool.

The cost of the NRA has been evaluated in three studies. In Norway, Syre *et al.*¹⁷ have estimated the price of the NRA performed in liquid media as 3 USD per isolate for two drugs, compared with 21 USD for the BACTEC 460 method and 23 USD for the manual mycobacterial growth indicator tube (MGIT). In India, Poojary *et al.*²⁵ have calculated the price for the NRA performed in liquid media, and the cost has been estimated at 145 Rs per isolate, which corresponds to 3.7 USD including media and reagents but not salary and infrastructure required for performing the test compared with 45 Rs, which is around 1.13 USD, for the conventional method. In Argentina, Mengatto *et al.*²⁶ compared the price to test one isolate for two drugs and estimated 19.52 USD for the manual MGIT and 0.17 USD for the NRA. This does not include labour costs.

Conclusions

The NRA has been shown to be highly sensitive and specific in the detection of rifampicin and isoniazid resistance when used on clinical isolates. With the objective to reduce the TTP,

the NRA performed directly on sputum smear-positive samples saves valuable time by omitting the pre-isolation step. Additional research is required to better establish the accuracy of the NRA applied directly on sputum samples, as very few studies are reported in the literature. However, available data from these studies show that the NRA is promising to be applied directly on sputum samples. Studies are also required to determine the performance of the test in countries with a high prevalence of MDR-TB or in a population in which MDR-TB is suspected. Finally, additional studies are required to establish the cost-effectiveness of the NRA compared with the conventional methods to demonstrate the benefit of this technology.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

References

1. Shah NS, Wright A, Bai GH *et al.* Worldwide emergence of extensively drug-resistant tuberculosis. *Emerg Infect Dis* 2007; **13**: 380–7.
2. Aziz MA, Wright A, Laszlo A *et al.* WHO/International Union Against Tuberculosis and Lung Disease Global Project on Anti-tuberculosis Drug Resistance Surveillance. Epidemiology of anti-tuberculosis drug resistance (the Global Project on Anti-tuberculosis Drug Resistance Surveillance): an updated analysis. *Lancet* 2006; **368**: 2142–54.
3. World Health Organization. The global MDR-TB and XDR-TB response plan. WHO/HTM/TB/2007.387. Geneva: World Health Organization, 2007.
4. 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 programs. *Bull World Health Org* 1969; **41**: 21–43.
5. Canetti G, Froman S, Grosset J *et al.* Mycobacteria: laboratory methods for testing drug sensitivity and resistance. *Bull World Health Org* 1963; **29**: 565–78.

Systematic review

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. Palomino JC. Newer diagnostics for tuberculosis and multi-drug resistant tuberculosis. *Curr Opin Pulm Med* 2006; **12**: 172–8.
8. Griess P. Bemerkungen zu der Abhandlung der HH. Weselky und Benedikt Ueber eineige Azoverbindungen. *Berichte der Deutschen Chemischen Gesellschaft* 1879; **12**: 426–8.
9. Walter SD. The partial area under the summary ROC curve. *Stat Med* 2005; **24**: 2025–40.
10. Walter SD. Properties of the summary receiver operating characteristic (SROC) curve for diagnosis test data. *Stat Med* 2002; **21**: 1237–56.
11. 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.
12. Huedo-Medina TB, Sánchez-Meca J, Marín-Martínez F *et al*. Assessing heterogeneity in meta-analysis: Q statistic or I^2 index? *Psychol Methods* 2006; **11**: 193–206.
13. Song F, Khan KS, Dinnes J *et al*. Asymmetric funnel plots and publication bias in meta-analyses of diagnostic accuracy. *Int J Epidemiol* 2002; **31**: 88–95.
14. Egger M, Davey Smith G, Schneider M *et al*. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629–34.
15. 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.
16. Angeby KA, Klintz L, Hoffner SE. Rapid and inexpensive drug susceptibility testing of *Mycobacterium tuberculosis* with a nitrate reductase assay. *J Clin Microbiol* 2002; **40**: 553–5.
17. Syre H, Phyu S, Sandven P *et al*. Rapid colorimetric method for testing susceptibility of *Mycobacterium tuberculosis* to isoniazid and rifampin in liquid cultures. *J Clin Microbiol* 2003; **41**: 5173–7.
18. 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.
19. Coban AY, Birinci A, Ekinci B *et al*. Drug susceptibility testing of *Mycobacterium tuberculosis* with nitrate reductase assay. *Int J Antimicrob Agents* 2004; **24**: 304–6.
20. Sethi S, Sharma S, Sharma SK *et al*. Drug susceptibility of *Mycobacterium tuberculosis* to primary antitubercular drugs by nitrate reductase assay. *Indian J Med Res* 2004; **120**: 468–71.
21. Montoro E, Lemus D, Echemendia M *et al*. Comparative evaluation of the nitrate reduction assay, the MTT test, and the resazurin microtiter assay for drug susceptibility testing of clinical isolates of *Mycobacterium tuberculosis*. *J Antimicrob Chemother* 2005; **55**: 500–5.
22. Martin A, Montoro E, Lemus D *et al*. Multicenter evaluation of the nitrate reductase assay for drug resistance detection of *Mycobacterium tuberculosis*. *J Microbiol Methods* 2005; **63**: 145–50.
23. 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.
24. Kumar M, Khan IA, Verma V *et al*. Microplate nitrate reductase assay versus Alamar Blue assay for MIC determination of *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis* 2005; **9**: 939–41.
25. Poojary A, Nataraj G, Kanade S *et al*. Rapid antibiotic susceptibility testing of *Mycobacterium tuberculosis*: its utility in resource poor settings. *Indian J Med Microbiol* 2006; **24**: 268–72.
26. Mengatto L, Chiani Y, Imaz MS. Evaluation of rapid alternative methods for drug susceptibility testing in clinical isolates of *Mycobacterium tuberculosis*. *Mem Inst Oswaldo Cruz* 2006; **101**: 535–42.
27. Lemus D, Montoro E, Echemendia M *et al*. Nitrate reductase assay for detection of drug resistance in *Mycobacterium tuberculosis*: simple and inexpensive method for low-resource laboratories. *J Med Microbiol* 2006; **55**: 861–3.
28. Musa HR, Ambroggi M, Souto A *et al*. Drug susceptibility testing of *Mycobacterium tuberculosis* by a nitrate reductase assay applied directly on microscopy-positive sputum samples. *J Clin Microbiol* 2005; **43**: 3159–61.
29. Solis LA, Shin SS, Han LL *et al*. Validation of a rapid method for detection of *Mycobacterium tuberculosis* resistance to isoniazid and rifampin in Lima, Peru. *Int J Tuberc Lung Dis* 2005; **9**: 760–4.
30. Affolabi D, Odoun M, Martin A *et al*. Evaluation of direct detection of *Mycobacterium tuberculosis* rifampin resistance by a nitrate reductase assay applied to sputum samples in Cotonou, Benin. *J Clin Microbiol* 2007; **45**: 2123–5.
31. Panaiotov S, Kantardjiev T. Nitrate reductase assay for drug susceptibility testing of *Mycobacterium tuberculosis*. *J Clin Microbiol* 2002; **40**: 3881–2.
32. Golyshevskaja VI, Irtuganova OA, Smirnova NS *et al*. Comparison of nitrate reductase and automatic BACTEC MGIT 960 AST techniques for determining the drug sensitivity of *Mycobacterium tuberculosis*. *Probl Tuberk Bolezn Legk* 2003; **8**: 34–7.
33. Poliakov AE, Safonova SG, Skotnikova OI. Determination of multidrug resistance of *M. tuberculosis* by different methods. *Probl Tuberk Bolezn Legk* 2006; **6**: 40–2.