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Review

Resazurin microtiter assay for isoniazid, rifampicin, ethambutol and streptomycin resistance detection in *Mycobacterium tuberculosis*: Updated meta-analysis

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ABSTRACT

Aims: The present meta-analysis aims to assess the evidence regarding the diagnostic accuracy and performance characteristics of the colorimetric redox indicator (CRI) assay with a special emphasis on the use of the resazurin microtiter assay (REMA) for determination of primary anti-tuberculosis drug resistance.

Subject and methods: By updating previous literature searches in Medline PubMed, ISI Web, Web of Science and Google academic databases of the REMA test for determination of primary anti-tuberculosis drug resistance, this meta-analysis includes 14 studies for isoniazid (INH); 15 studies for rifampicin (RIF); 6 studies for streptomycin (STR); and 5 studies for ethambutol (EMB). SROC curve analysis was performed for meta-analysis and diagnostic accuracy was summarized.

Results: Pooled sensitivity was 96% (94–98%) for INH, 97% (95–98%) for RIF, 92% (87–96%) for EMB and 92% (88–95%) for STR. Pooled specificity for INH, RIF, EMB and STR was 96% (95–98%), 99% (98–99%), 86% (81–89%) and 90% (87–93%), respectively. Susceptibility testing results had been obtained in 8–9 days.

Conclusion: In conclusion, REMA seems to be a reliable test for the determination of multidrug resistant (MDR) isolates in laboratories with limited resources. However, few studies for STR and EMB have been found, and cost-effectiveness studies need to be determined to recommend its widespread use.

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Contents

Introduction

Tuberculosis (TB) continues to be an important cause of morbidity and mortality worldwide [1]. The World Health Organization (WHO) reported that there were 8.7 million TB cases, and 13% of these were co-infected with the human immunodeficiency virus (HIV) in 2011. It also reported that TB caused 1.4 million deaths [2]. Increased multi-drug resistant TB (MDR-TB) cases threaten a more effective control of the disease [3]. Rapid and accurate identification of these resistant cases is the most important step for preventing the spread of MDR-TB isolates in the population. Conventional methods for drug susceptibility testing (DST) including proportion, absolute concentration and resistant ratio methods are used for identification of drug-resistant isolates. Rapid automated systems (Bactec 460 TB and Bactec MGIT 960) are also available for this purpose [4,5]. However, obtaining DST results on conventional Löwenstein–Jensen or agar-based media requires 3–6 weeks. Automated systems are faster, but their high cost and need for equipment limit their use in developing countries for early diagnosis and management of MDR-TB [3].

Table 1 – Description of studies included in the meta-analysis of INH resistance detection.

References	Countries	Reference test	No. isolates	Sensitivity (95% CI)	Specificity (95% CI)	TTP (day)
Dixit et al. [12]	India	PM on LJ	105	0.93 (0.82–0.98)	0.98 (0.89–1.00)	8
Bwanga et al. [14]	Uganda/Sweden	PM on LJ	31	0.88 (0.64–0.99)	0.57 (0.29–0.82)	8
Montoro et al. [15]	Cuba/Belgium	PM on LJ	100	1.00 (0.92–1.00)	0.96 (0.87–1.00)	8
Palomino et al. [16]	Belgium/Bolivia/Peru	PM on LJ	80	1.00 (0.93–1.00)	0.96 (0.80–1.00)	8
Martin et al. [10]						
Site 1	Belgium	PM on LJ	30	1.00 (0.74–1.00)	1.00 (0.81–1.00)	8
Site 2	Argentina	PM on LJ	30	1.00 (0.74–1.00)	1.00 (0.81–1.00)	8
Site 3	Cuba	PM on LJ	30	1.00 (0.74–1.00)	0.94 (0.73–1.00)	8
Site 4	Brazil	PM on LJ	30	1.00 (0.74–1.00)	0.94 (0.73–1.00)	8
Site 5	Colombia	PM on LJ	30	1.00 (0.69–1.00)	1.00 (0.75–1.00)	8
Site 6	Chile	PM on LJ	30	0.92 (0.62–1.00)	1.00 (0.81–1.00)	8
Site 7	Nicaragua	PM on LJ	30	0.92 (0.62–1.00)	0.94 (0.73–1.00)	8
Rivoire et al. [17]	Madagascar/Belgium	PM on LJ	77	0.95 (0.83–0.99)	0.97 (0.86–1.00)	8
Affolabi et al. [18]	Benin/Belgium/France	PM on LJ	151	1.00 (0.85–1.00)	1.00 (0.97–1.00)	8
Rondon et al. [19]	Venezuela/USA	PM on 7H10 agar	155	0.79 (0.66–0.89)	0.96 (0.90–0.99)	8
Martin et al. [20]	Belgium/Argentina/ Colombia/Sweden	PM on LJ	149	0.99 (0.95–1.00)	1.00 (0.88–1.00)	8
Coban et al. [11]	Turkey	Bactec 460 TB	50	1.00 (0.87–1.00)	0.92 (0.73–0.99)	8–9
Iglesia et al. [21]	Argentina	PM on 7H11 agar	69	1.00 (0.85–1.00)	0.94 (0.82–0.99)	8
Coban et al. [22]	Turkey	Bactec 460 TB/MGIT 960	73	1.00 (0.89–1.00)	0.90 (0.77–0.97)	8
Nateche et al. [23]	Algeria/Belgium	PM on LJ	136	1.00 (0.80–1.00)	0.98 (0.94–1.00)	8
Banfi et al. [24]	Italy	PM on 7H11 agar	13	1.00 (0.48–1.00)	1.00 (0.63–1.00)	8

TTP: Time to positivity.

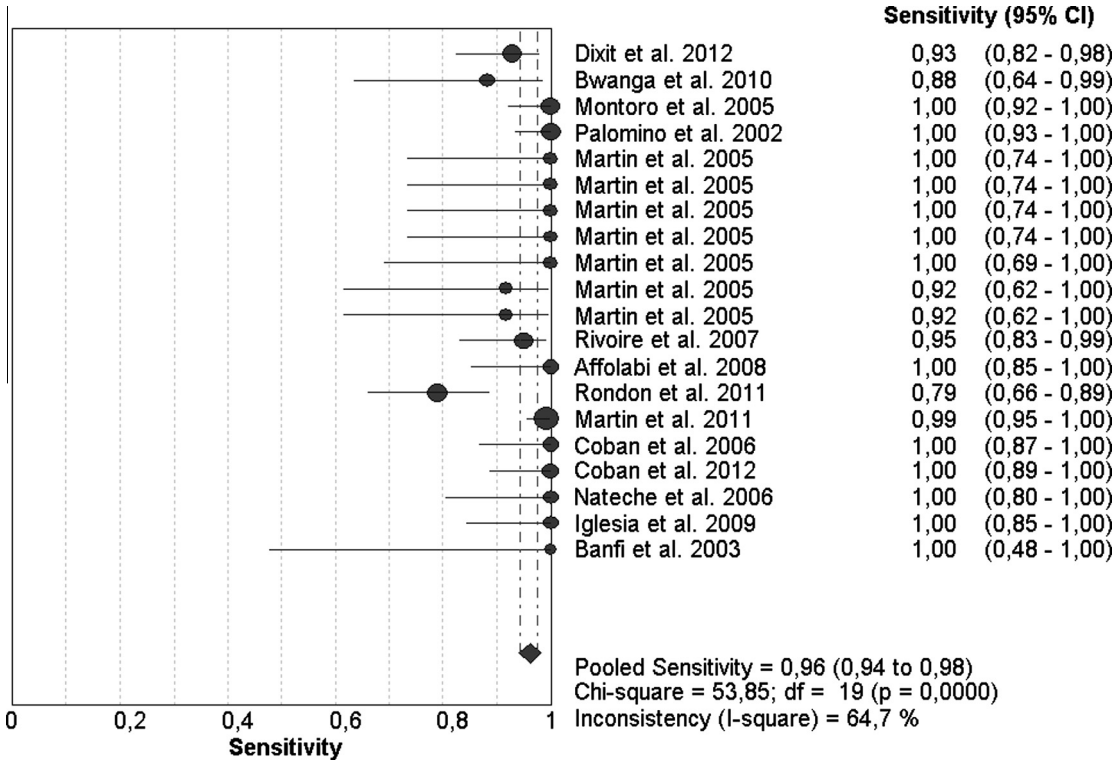

Fig. 1 – Forest plot of the sensitivity for INH assay. The point estimates of sensitivity from each study are shown as circles. Error bars 95% confidence intervals.

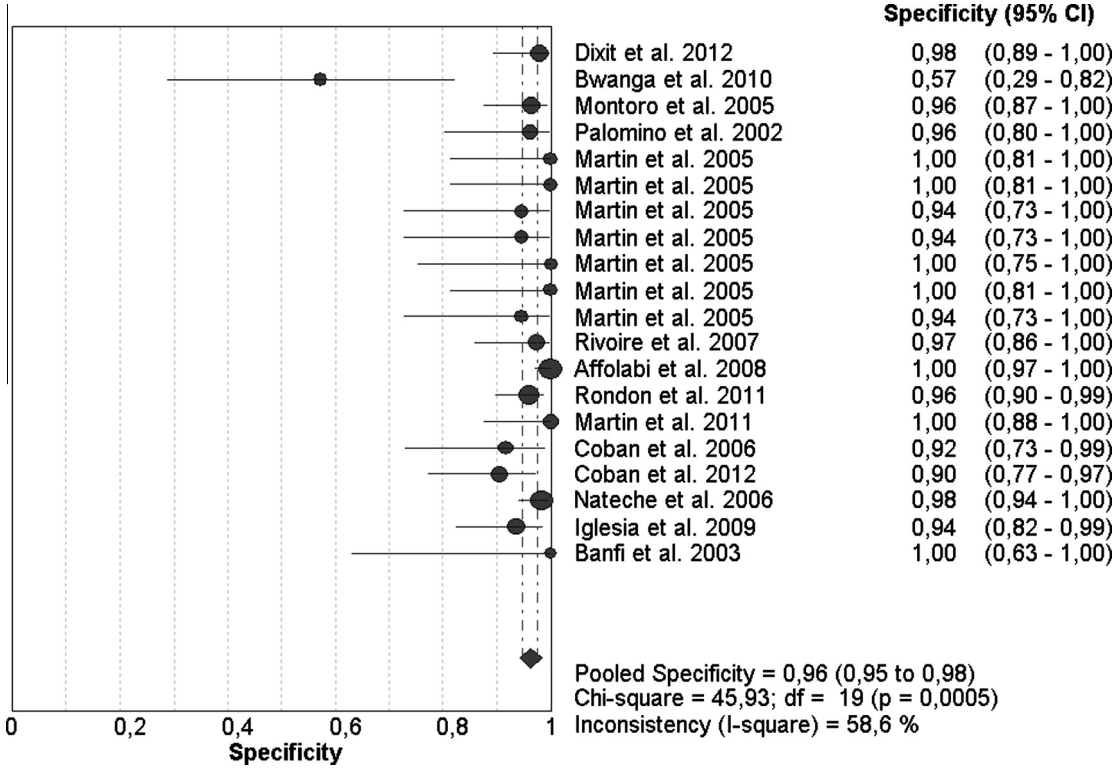

Fig. 2 – Forest plot of the specificity for INH assay. The point estimates of specificity from each study are shown as circles. Error bars 95% confidence intervals.

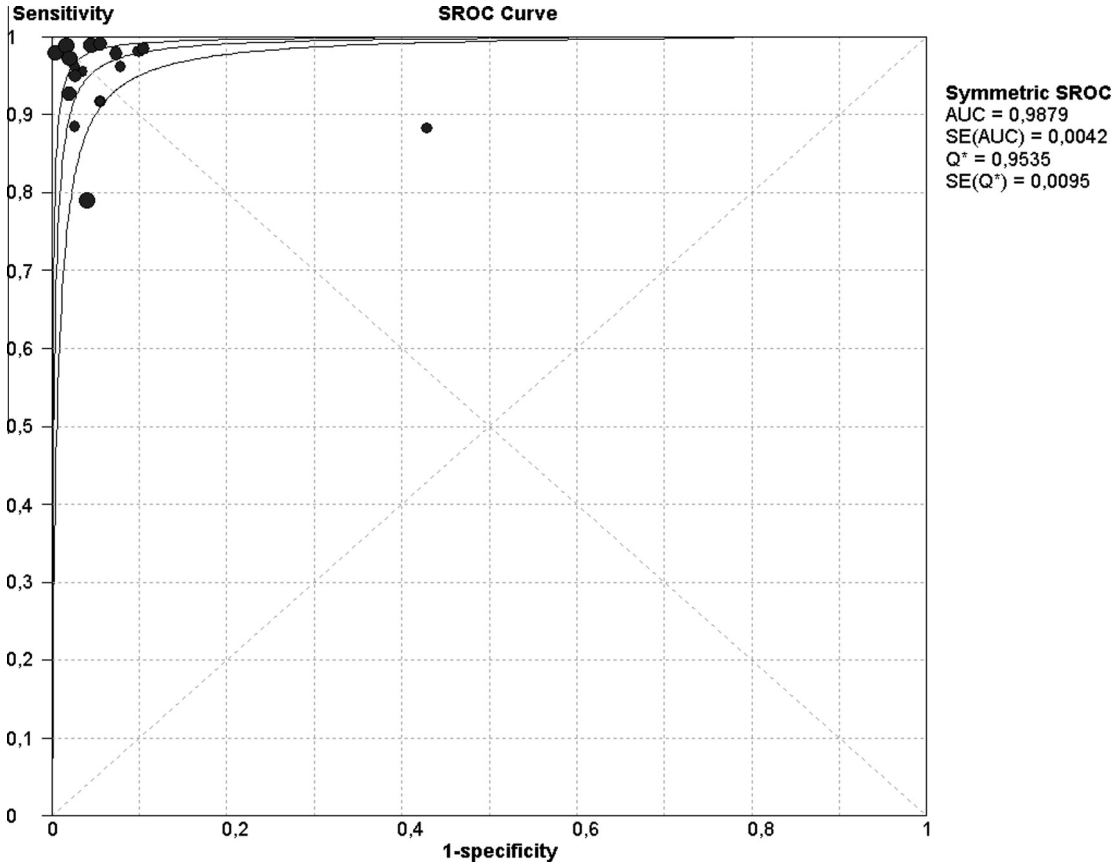

Fig. 3 – Summary receiver operating characteristic (SROC) plot for INH. Each circle represents each study in the analysis. The curve is the regression line that summarizes the overall diagnostic accuracy. SROC, summary receiver operating characteristic; AUC, area under the curve; SE (AUC), standard error 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.

In 2007, a systematic review and meta-analysis was performed synthesizing the evidence available on the accuracy of the colorimetric method for detecting rifampicin (RIF) and isoniazid (INH) resistance [3]. Evidence summarized in that review did not include streptomycin (STR) or ethambutol (EMB). Since 2007, there have been several new studies looking not only at RIF and INH, but also all first-line drugs.

Resazurin microtiter assay (REMA), a rapid, reliable, inexpensive and easily performed colorimetric method, has gained interest recently for the determination of MDR-TB. Additionally, it is recommended among non-commercial DST methods by the WHO [6].

The objective of this study was to update the previously reported systematic review and meta-analysis of evidence regarding the diagnostic accuracy and performance characteristics of the REMA test for determination of primary antituberculosis drugs, including INH, RIF, EMB and STR resistance, among *Mycobacterium tuberculosis*.

Materials and methods

Literature search

Medline PubMed, ISI Web, Web of Science and Google academic databases were searched using the keywords "resazurin" "resazurin microtiter assay", "resazurin assay", "colorimetric", "*Mycobacterium tuberculosis*", "tuberculosis", "drug susceptibility", "drug resistance", "isoniazid", "rifampicin", "ethambutol" and "streptomycin". Studies published up to June 2013 in English, French, or Spanish and those that met the inclusion criteria were identified and were included in the meta-analysis.

Inclusion criteria

Studies were included that compared REMA with a reference method using a solid culture, such as Löwenstein–Jensen or Middlebrook 7H10/7H11 agar medium, or using a liquid culture, such as BACTEC MGIT 960, and studies that data could be extracted with diagnostic accuracy. Studies reported in conference abstract books were excluded.

Data extraction

The retrieved articles were reviewed by two independent reviewers. Titles and abstracts of articles were read, and eligible ones were marked for data extraction. Two reviewers extracted data independently including author, year of publication of the study, country in which the study was performed, reference method used (indirect or direct),

Table 2 – Description of studies included in the meta-analysis of RIF resistance detection.

References	Countries	Reference test	No. isolates	Sensitivity (%95 CI)	Specificity (%95 CI)	TTP (day)
Dixit et al. [12]	India	PM on LJ	105	0.95 (0.85–0.99)	1.00 (0.93–1.00)	8
Bwanga et al. [14]	Uganda/Sweden	PM on LJ	31	0.71 (0.42–0.92)	0.94 (0.71–1.00)	8
Montoro et al. [15]	Cuba/Belgium	PM on LJ	100	1.00 (0.91–1.00)	0.98 (0.91–1.00)	8
Palomino et al. [16]	Belgium/Bolivia/Peru	PM on LJ	80	1.00 (0.93–1.00)	1.00 (0.89–1.00)	8
Martin et al. [10]						
Site 1	Belgium	PM on LJ	30	1.00 (0.78–1.00)	1.00 (0.78–1.00)	8
Site 2	Argentina	PM on LJ	30	1.00 (0.78–1.00)	1.00 (0.78–1.00)	8
Site 3	Cuba	PM on LJ	30	0.93 (0.68–1.00)	1.00 (0.78–1.00)	8
Site 4	Brazil	PM on LJ	30	1.00 (0.78–1.00)	1.00 (0.78–1.00)	8
Site 5	Colombia	PM on LJ	30	1.00 (0.74–1.00)	1.00 (0.72–1.00)	8
Site 6	Chile	PM on LJ	30	1.00 (0.78–1.00)	1.00 (0.78–1.00)	8
Site 7	Nicaragua	PM on LJ	30	0.93 (0.68–1.00)	0.93 (0.68–1.00)	8
Rivoire et al. [17]	Madagascar/Belgium	PM on LJ	77	0.95 (0.75–1.00)	1.00 (0.94–1.00)	8
Affolabi et al. [18]	Benin/Belgium/France	PM on LJ	151	0.94 (0.70–1.00)	1.00 (0.97–1.00)	8
Rondon et al. [19]	Venezuella/USA	PM on 7H10 agar	155	0.80 (0.59–0.93)	0.98 (0.93–1.00)	8
Martin et al. [20]	Belgium/Argentina/ Colombia/Sweden	PM on LJ	149	1.00 (0.97–1.00)	0.94 (0.81–0.99)	8
Coban et al. [11]	Turkey	Bactec 460 TB	50	1.00 (0.81–1.00)	1.00 (0.89–1.00)	8–9
Iglesia et al. [21]	Argentina	PM on 7H11 agar	69	1.00 (0.86–1.00)	0.98 (0.88–1.00)	8
Coban et al. [22]	Turkey	Bactec 460 TB/MGIT 960	73	0.91 (0.72–0.99)	1.00 (0.93–1.00)	8
Nateche et al. [23]	Algeria/Belgium	PM on LJ	136	1.00 (0.74–1.00)	0.99 (0.96–1.00)	8
Banfi et al. [24]	Italy	PM on 7H11 agar	13	1.00 (0,39–1.00)	1.00 (0.66–1.00)	9
Lemus et al. [25]	Cuba/Belgium	PM on LJ	20	1.00 (0.69–1.00)	1.00 (0.69–1.00)	8

TTP: Time to positivity.

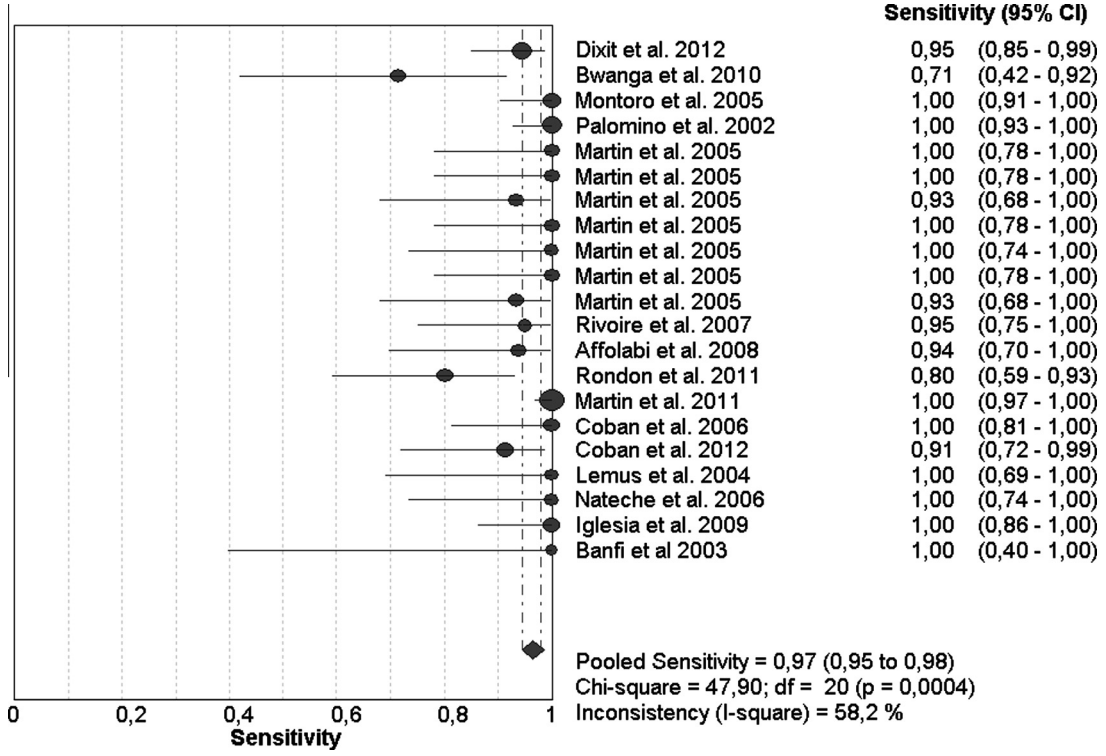

Fig. 4 – Forest plot of the sensitivity for RIF analysis. The point estimates of sensitivity from each study are shown as circles. Error bars 95% confidence intervals.

number of samples, sensitivity, specificity and time for obtaining results; all information gathered was then entered into a datasheet of Microsoft Excel software. Disagreements between reviewers were solved by consensus. Sensitivity [true positive rate (TPR)] was defined as the proportion of isolates determined to be resistant by the reference method and correctly identified as resistant by REMA. Specificity [true negative rate (TNR)] was defined as the proportion of isolates determined to be susceptible by the reference method and correctly identified as susceptible by REMA.

Meta-analysis

Meta-analysis was performed by using Meta-DiSc software (version 1.4) [7]. It was focused on the sensitivity and specificity values for measurement of diagnostic accuracy of the used test. A forest plot was created for estimating the accuracy of the test. Receiver operating characteristic (ROC) curves indicating the relationship between the TPR and the false positive rate (FPR) of the test were also plotted. The Q^* index represents a summarization of the test performance where sensitivity and specificity are equal. The heterogeneity X^2 and I^2 indexes were used for analyzing the heterogeneity among studies used for meta-analysis.

Quality assessment

The quality of individual studies was assessed by using criteria based on the QUADAS tools for the assessment of quality of diagnostic studies [8].

Reporting bias

Publication bias using methods such as funnel plots or regression tests were not performed in this review because such techniques have not been found to be helpful for diagnostic studies [9].

Results

Detection of INH resistance

Data including country in which the study was performed, reference method used in the study, number and type of samples, sensitivity and specificity values and time for obtaining results in which REMA was used for determination of INH resistance are summarized in Table 1. Fourteen studies were included and analyzed. One study [10] was performed in 7 different centers, so the results of each center are presented individually in Table 1. REMA was performed as an indirect test among clinical isolates in all studies. The reference test was the propor-

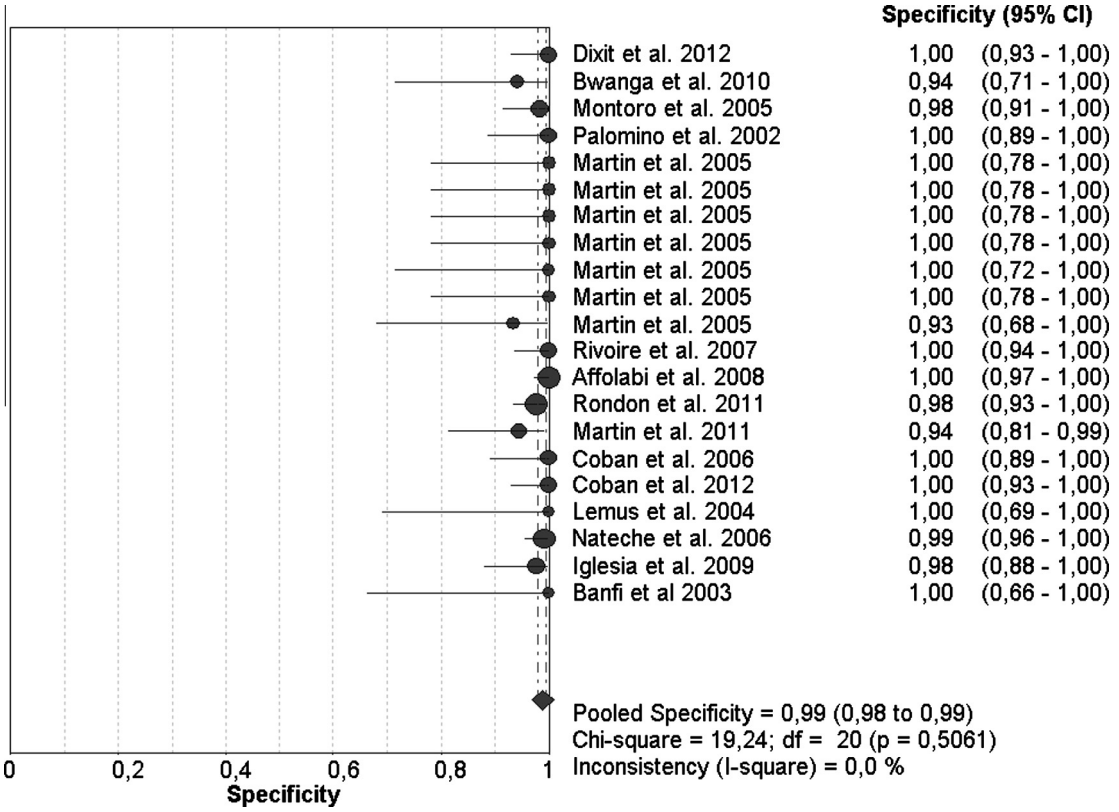

Fig. 5 – Forest plot of the specificity for RIF analysis. The point estimates of specificity from each study are shown as circles. Error bars 95% confidence intervals.

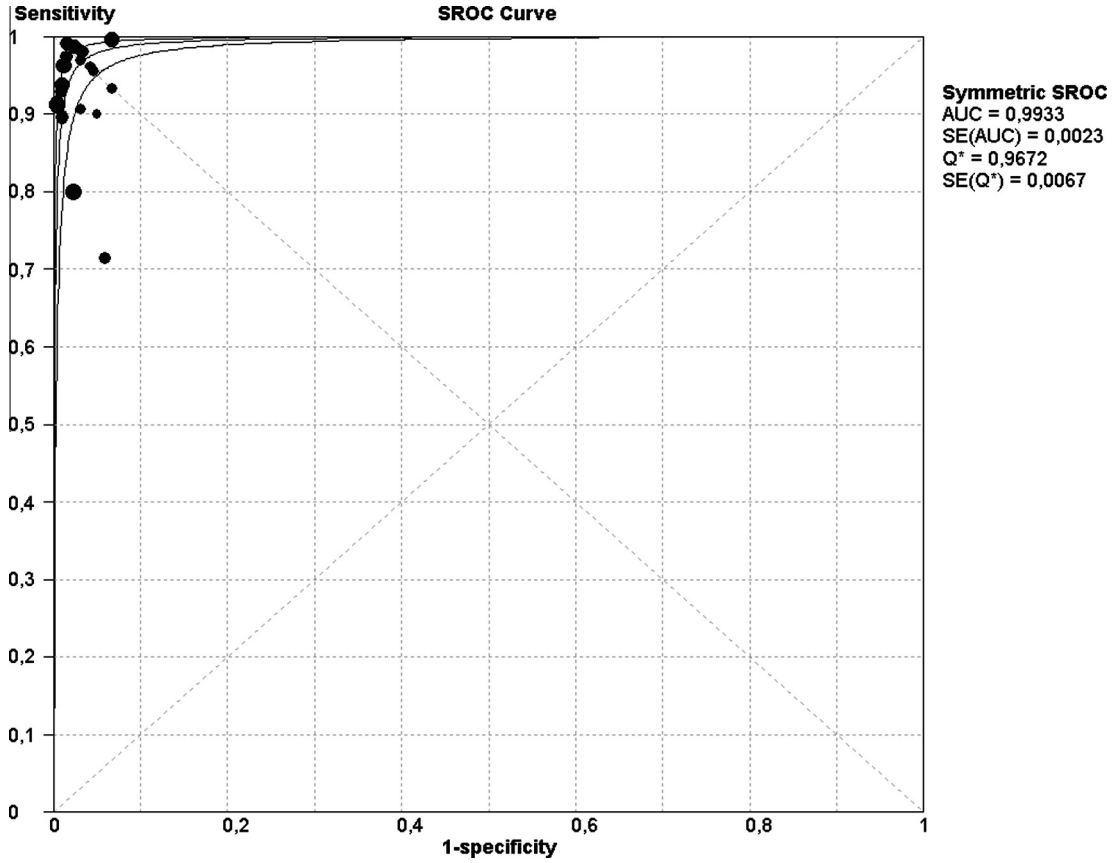

Fig. 6 – Summary receiver operating characteristic (SROC) plot for RIF. Each circle represents each study in the analysis. The curve is the regression line that summarizes the overall diagnostic accuracy. SROC, summary receiver operating characteristic; AUC, area under the curve; SE (AUC), standard error 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.

Table 3 – Description of studies included in the meta-analysis of EMB resistance detection.

References	Countries	Reference test	No. isolates	Sensitivity (%95 CI)	Specificity (%95 CI)	TTP (day)
Dixit et al. [12]	India	PM on LJ	105	0.84 (0.71–0.92)	0.94 (0.83–0.99)	8
Montoro et al. [15]	Cuba/Belgium	PM on LJ	100	0.94 (0.71–1.00)	0.58 (0.46–0.69)	8
Martin et al. [10]						
Site 1	Belgium	PM on LJ	30	0.89 (0.52–1.00)	0.95 (0.76–1.00)	8
Site 2	Argentina	PM on LJ	30	1.00 (0.66–1.00)	1.00 (0.84–1.00)	8
Site 3	Cuba	PM on LJ	30	1.00 (0.66–1.00)	0.95 (0.76–1.00)	8
Site 4	Brazil	PM on LJ	30	1.00 (0.66–1.00)	0.95 (0.76–1.00)	8
Site 5	Colombia	PM on LJ	30	1.00 (0.63–1.00)	1.00 (0.78–1.00)	8
Site 6	Chile	PM on LJ	30	1.00 (0.66–1.00)	1.00 (0.84–1.00)	8
Site 7	Nicaragua	PM on LJ	30	0.89 (0.52–1.00)	0.90 (0.68–0.99)	8
Banfi et al. [24]	Italy	PM on 7H11 agar	13	1.00 (0.39–1.00)	1.00 (0.66–1.00)	9
Jadaun et al. [26]	India	PM on LJ	50	0.97 (0.83–1.00)	1.00 (0.82–1.00)	8–9

TTP: Time to positivity.

tion method (PM) on Löwenstein–Jensen (LJ) medium in 9 studies; Bactec 460 TB in 2 studies; PM on 7H11 agar in 2 studies; PM on 7H10 agar in 1 study; and MGIT 960 in 1 study.

REMA was performed in microtiter plates in 13 of 14 studies, whereas it was performed in tubes with screw caps as a macro test in one study [11]. Furthermore, the test was performed in albumin-containing Dubos broth in 1 study; in 7H9 broth in 3 studies; and 7H9-S (with 0.1% casitone) broth in the remaining 10 studies.

Figs. 1 and 2 illustrate forest plots that estimate the sensitivity and specificity based on results of the 14 included studies. Fig. 3 is an SROC curve of the same data. Sensitivity was determined as 88–100% and specificity was reported as 57–100% (Table 1, Figs. 1 and 2). The SROC curve shows an

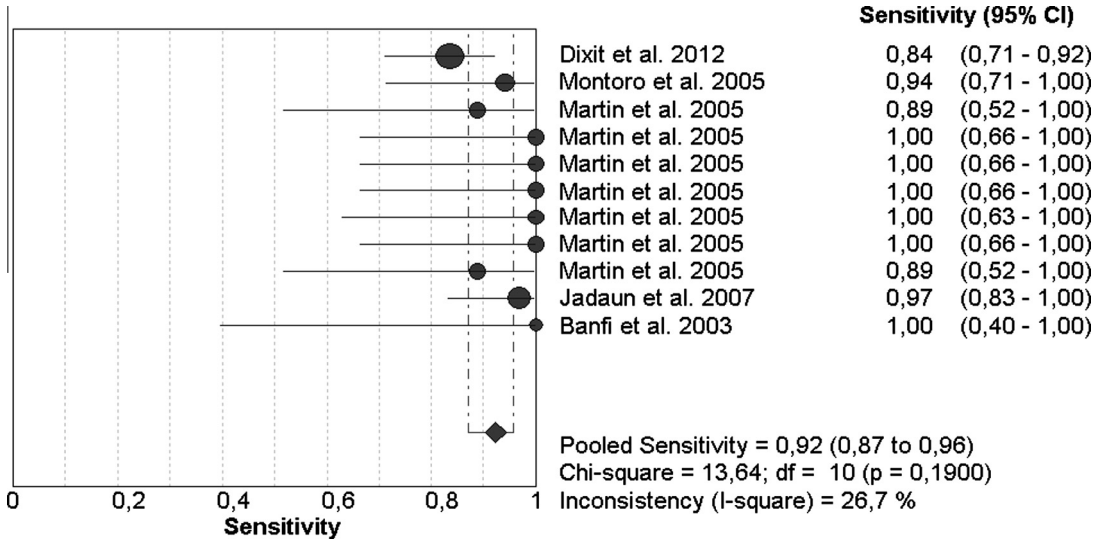

Fig. 7 – Forest plot of the sensitivity for EMB. The point estimates of sensitivity from each study are shown as circles. Error bars 95% confidence intervals.

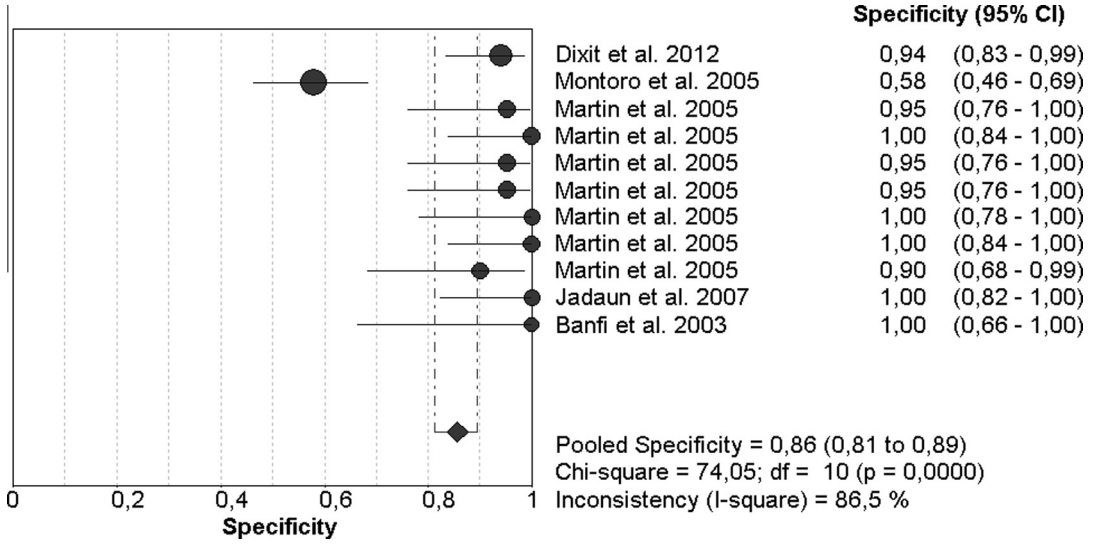

Fig. 8 – Forest plot of the specificity for EMB. The point estimates of specificity from each study are shown as circles. Error bars 95% confidence intervals.

AUC of 0.98 and Q* of 0.95, indicating a high level of overall accuracy (Fig. 3).

Detection of RIF resistance

Results of studies in which REMA was used for determination of RIF resistance are summarized in Table 2. Fifteen studies were analyzed. Results were evaluated individually and are presented in Table 2. All studies evaluated the test with clinical isolates. The reference method was PM on LJ medium in 10 studies; Bactec 460 TB in 1 study; both Bactec 460 TB and Bactec MGIT 960 in 1 study; PM on 7H10 agar in 1 study; and PM on 7H11 agar in 2 studies.

REMA was performed in microtiter plates in 14 of 15 analyzed studies, whereas it was performed as a macro test in tubes with a screw cap in 1 study. It was also performed in albumin-containing Dubos broth in 1 study; in 7H9 broth in 3 studies; and in 7H9-S broth in the remaining 10 studies.

Figs. 4 and 5 illustrate forest plots that estimate the sensitivity and specificity based on results of the 14 included studies. Fig. 6 is an SROC curve of the same data. Sensitivity was determined as 71–100% and specificity was 93.3–100% (Table 2, Figs. 4 and 5). The SROC curve shows an AUC of 0.99 and Q* of 0.96, indicating a high level of overall accuracy (Fig. 6).

Detection of EMB resistance

Results of studies in which REMA was used for determination of EMB resistance are summarized in Table 3. Only 5 studies

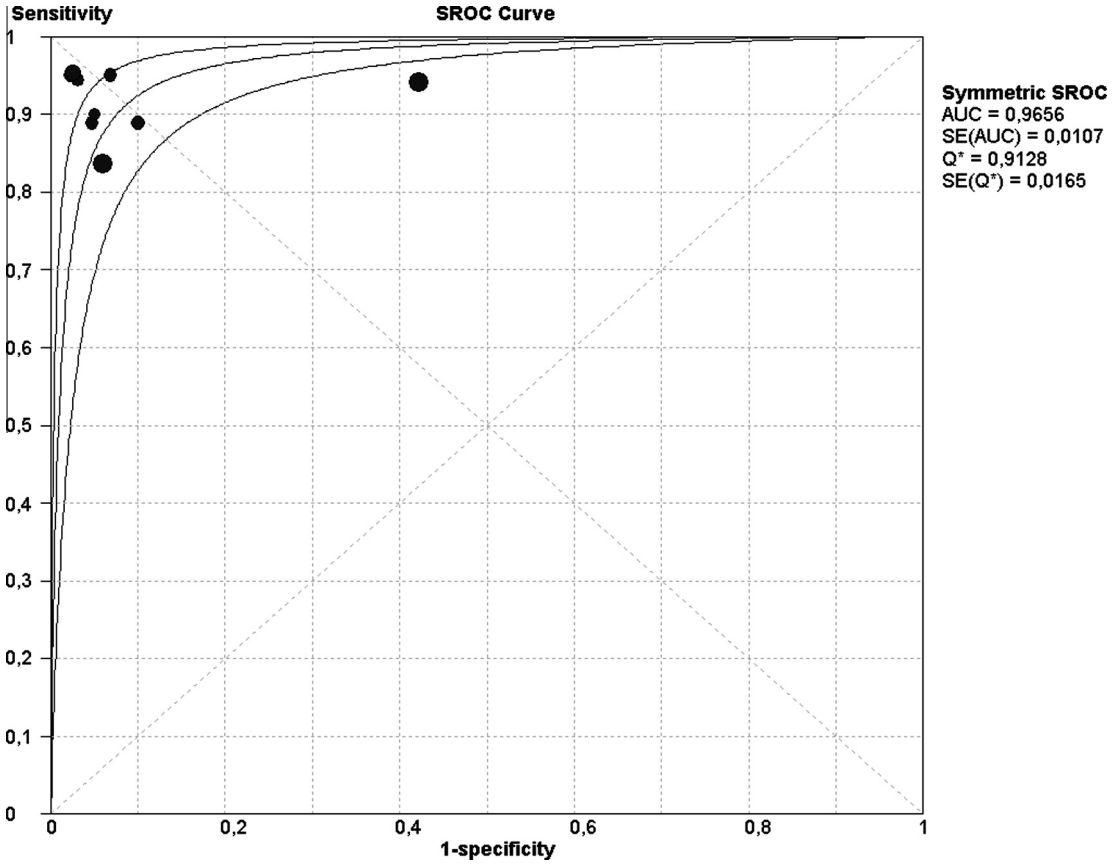

Fig. 9 – Summary receiver operating characteristic (SROC) plot for EMB. Each circle represents each study in the analysis. The curve is the regression line that summarizes the overall diagnostic accuracy. SROC, summary receiver operating characteristic; AUC, area under the curve; SE (AUC), standard error 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.

Table 4 – Description of studies included in the meta-analysis of STR resistance detection.

References	Countries	Reference test	No. isolates	Sensitivity (%95 CI)	Specificity (%95 CI)	TTP (day)
Dixit et al. [12]	India	PM on LJ	105	0.87 (0.76–0.95)	0.96 (0.86–1.00)	8
Montoro et al. [15]	Cuba/Belgium	PM on LJ	100	0.94 (0.83–0.99)	0.88 (0.77–0.96)	8
Martin et al. [10]						
Site 1	Belgium	PM on LJ	30	0.83 (0.52–0.98)	0.72 (0.47–0.90)	8
Site 2	Argentina	PM on LJ	30	1.00 (0.74–1.00)	0.83 (0.59–0.96)	8
Site 3	Cuba	PM on LJ	30	0.92 (0.62–1.00)	0.78 (0.52–0.94)	8
Site 4	Brazil	PM on LJ	30	0.75 (0.43–0.95)	1.00 (0.81–1.00)	8
Site 5	Colombia	PM on LJ	30	1.00 (0.66–1.00)	0.79 (0.49–0.95)	8
Site 6	Chile	PM on LJ	30	0.92 (0.62–1.00)	0.83 (0.59–0.96)	8
Site 7	Nicaragua	PM on LJ	30	0.92 (0.62–1.00)	0.83 (0.59–0.96)	8
Rondón et al. [19]	Venezuella/USA	PM on 7H10 agar	155	0.92 (0.82–0.97)	0.96 (0.89–0.99)	8
Iglesia et al. [21]	Argentina	PM on 7H11 agar	69	1.00 (0.82–1.00)	0.94 (0.83–0.99)	8
Banfi et al. [24]	Italy	PM on 7H11 agar	13	1.00 (0.54–1.00)	1.00 (0.59–1.00)	9

TTP: Time to positivity.

were found for EMB and presented individually in Table 3. The test was performed as an indirect test with clinical isolates in all studies. PM on LJ was used as a reference test in 4 studies and PM on 7H11 agar in 1 study.

The test was performed in microtiter plates in all studies, and 7H9-S and 7H9 broth were used as media for the test in 4 and 1 study, respectively.

Figs. 7 and 8 illustrate forest plots that estimate the sensitivity and specificity based on results of the 4 included studies. Fig. 9 is a SROC curve of the same data. Sensitivity was 83.64–100%. However, the specificity was determined to be lower than for RIF and INH with a pooled specificity of 85%. Specificity was determined as 57.8–100% (Table 4, Figs. 7 and 8). The SROC curve shows an AUC of 0.96 and Q* of 0.91 (Fig. 9).

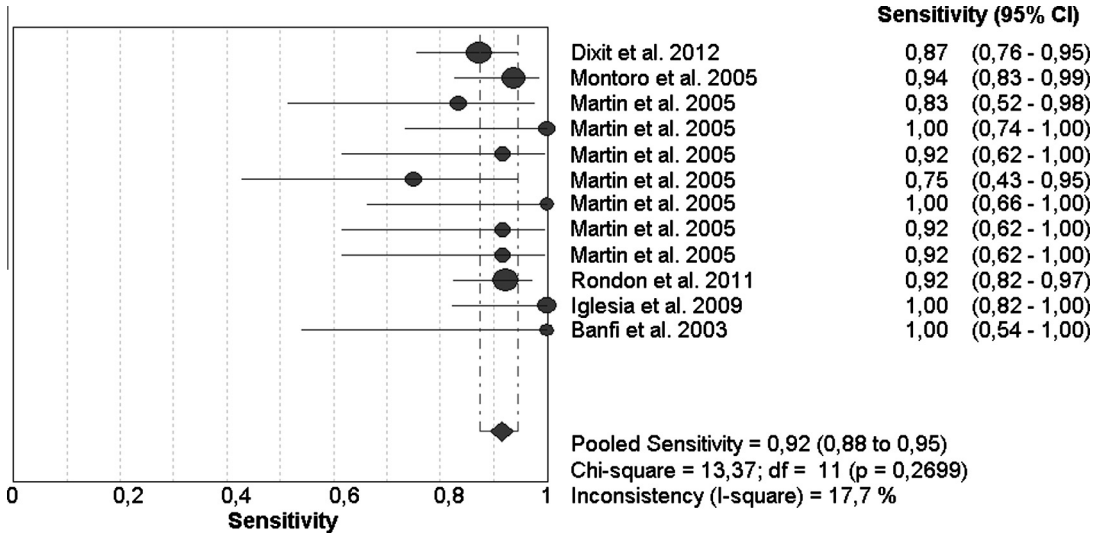

Fig. 10 – Forest plot of the sensitivity for STR. The point estimates of sensitivity from each study are shown as circles. Error bars 95% confidence intervals.

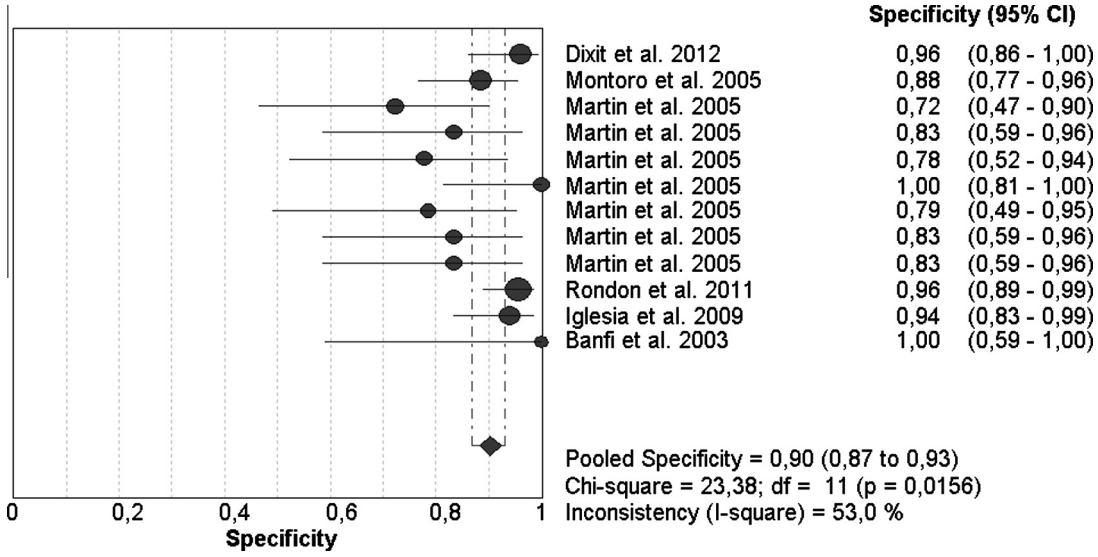

Fig. 11 – Forest plot of the specificity for STR. The point estimates of specificity from each study are shown as circles. Error bars 95% confidence intervals.

Detection of STR resistance

Six studies were analyzed for STR and are summarized in Table 4 individually. The test was performed as an indirect test among clinical isolates in all studies. The reference test was PM on LJ medium in 3 studies; PM on 7H10 agar in 1 study; and PM on 7H11 agar in 2 studies.

The REMA was performed in microtiter plates in all analyzed studies. It was also performed in 7H9-S broth in 4 studies and 7H9 broth in the remaining 2 studies.

Figs. 10 and 11 illustrate forest plots that estimate the sensitivity and specificity based on results of the 5 included studies. Fig. 12 is a SROC curve of the same data. Sensitivity was 75–100% and specificity was 72.2–100% (Table 4, Figs. 10 and 11). The SROC curve shows an AUC of 0.96 and Q* of 0.91 (Fig. 12).

The results were obtained in 8–9 days in all analyzed studies.

Discussion

One of the most important steps for better control of TB is the rapid determination of drug resistance and implementation of appropriate treatment [2,12]. There has been an increased interest in developing rapid, reliable and low-cost methods for this purpose. Colorimetric REMA is one of the proposed methods developed for this purpose.

This meta-analysis was aimed at evaluating REMA, which is a rapid, reliable and inexpensive method that gained an increased interest for the determination of primary anti-tuberculosis drug resistance. The results obtained indicate that the test has a high reliability for the determination of

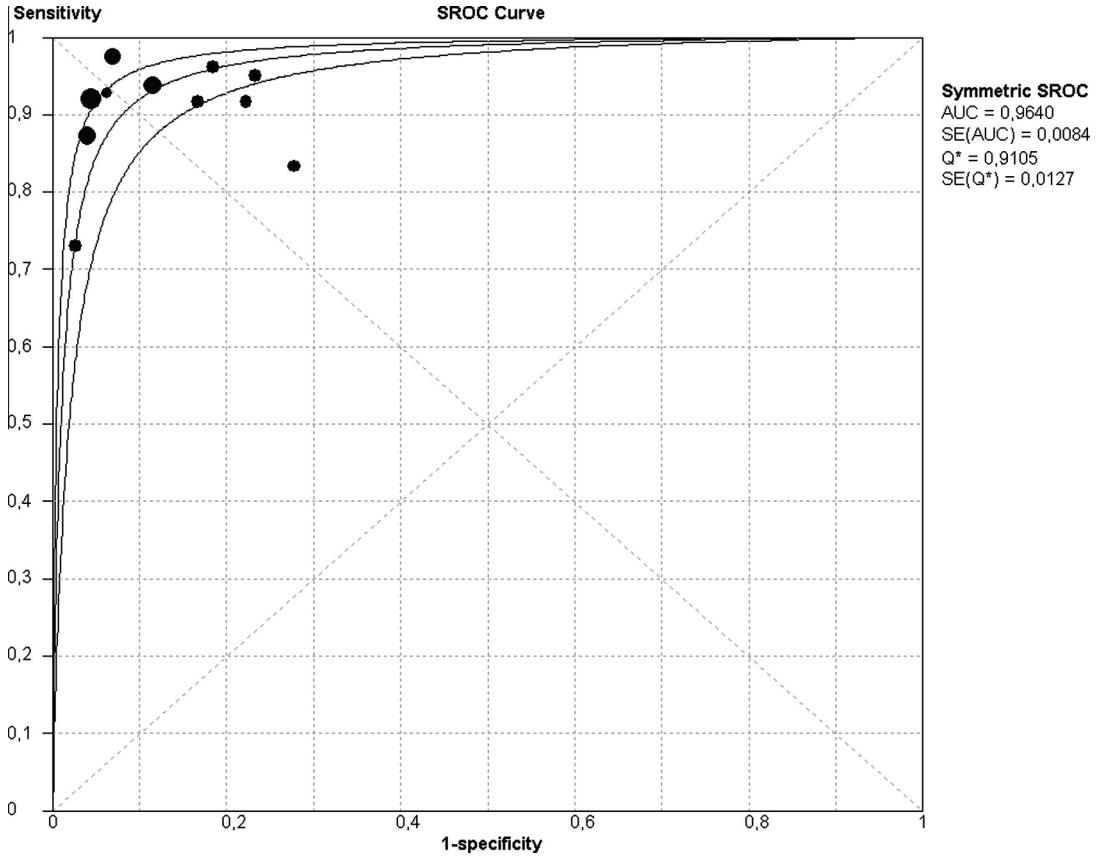

Fig. 12 – Summary receiver operating characteristic (SROC) plot for STR. Each circle represents each study in the analysis. The curve is the regression line that summarizes the overall diagnostic accuracy. SROC, summary receiver operating characteristic; AUC, area under the curve; SE (AUC), standard 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.

resistance to RIF and INH; however, lower values were obtained for EMB and STR. No definitive conclusions can be drawn for the latter two drugs since the number of published studies is still too low.

REMA was performed only indirectly among *M. tuberculosis* isolates grown in cultures, and as far as this research is considered, there is no study in which the test has been performed directly in sputum samples to detect drug resistance.

One meta-analysis on colorimetric redox indicator assays including REMA was found in the literature [3]. This previous meta-analysis evaluated the test only for determination of resistance to INH and RIF in 2007. It also analyzed the use of other indicators such as MTT or Alamar blue. That analysis included 6 studies using resazurin for INH and 7 for RIF [3]. The current meta-analysis is the first one specifically evaluating the REMA test for determination of all primary anti-tuberculosis drugs (INH, RIF, EMB and STR). The use of resazurin has become more frequent due to its low cost for viability testing. Moreover, the overall quality of the included studies was very good according to the analysis performed with the QUADAS tool.

The document "Noncommercial culture and drug susceptibility testing methods for screening patients at risk for multidrug-resistant tuberculosis" published by WHO defines that colorimetric redox indicator (CRI) assays like REMA can be used for the determination of drug resistance, but they are not faster than conventional phenotypic susceptibility tests performed in liquid media [6]. REMA, however, is much cheaper than phenotypic commercial methods. The cost of this method is estimated at approximately $3 for each strain tested against several drugs [13].

In conclusion, this meta-analysis showed that the REMA test is a reliable method for the detection of resistance to INH and RIF and has acceptable sensitivity and specificity for EMB and STR. However, more studies are needed to fully recommend its wide use.

Therefore, REMA can be used for early detection of MDR-TB cases, particularly in low-income countries due to its low cost and simple technology.

Conflict of interest

We have no conflict of interest to declare.

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