Pyrazinamide susceptibility testing in *Mycobacterium tuberculosis*: a systematic review with meta-analyses

Running title: Meta-analyses of pyrazinamide susceptibility testing in *M. tuberculosis*

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Abstract

Objectives: Standard culture-based susceptibility testing of *Mycobacterium tuberculosis* to pyrazinamide is difficult to perform. This systematic review with meta-analyses evaluated the role of molecular assays targeting *pncA* and pyrazinamidase assays.

Methods: PubMed and EMBASE were searched for relevant publications in English. Sensitivity and specificity were estimated in bivariate random effects models.

Results: Of 128 articles identified, 73 sets of data involving culture isolates were initially included in meta-analyses. Summary estimates of sensitivity and specificity were 87% and 93% for PCR-DNA sequencing (n=29), 75% and 95% for PCR-SSCP (n=5), 96% and 97% for a mixture of other molecular assays (n=6), and 89% and 97% for pyrazinamidase assays using the Wayne method (n=33). The median prevalence (range) of pyrazinamide resistance was 51% (31%-89%) in multidrug-resistant *M. tuberculosis* isolates, and 5% (0%-9%) in non-multidrug-resistant isolates. Excluding studies with possibly considerable false resistance in the reference assay gave the following estimates of sensitivity and specificity: 92% and 93% for PCR-DNA sequencing (n=20), 98% and 96% for other molecular assays (n=5), and 91% and 97% for the Wayne assay (n=27). With significant funnel plot asymmetry, the test performance of the Wayne assay might have been overestimated.

Conclusions: Considering the prevalence of pyrazinamide resistance in different clinical settings, PCR-DNA sequencing, and possibly other molecular assays targeting *pncA*, can detect pyrazinamide resistance in multidrug-resistant *M. tuberculosis* isolates with predictive values largely exceeding 90%, and rule out pyrazinamide resistance in non-multidrug-resistant isolates with predictive values exceeding 99%. Molecular assays for detecting pyrazinamide resistance are probably the way forward.
Keywords: drug susceptibility testing, pyrazinamide, molecular, resistant, tuberculosis.
Introduction

Multidrug-resistant tuberculosis (MDR-TB) including extensively drug-resistant tuberculosis (XDR-TB) has emerged as a global health threat (44, 71). In 2008, there were globally an estimated 390000-510000 cases of MDR-TB (71). The proportion of MDR-TB among all incident TB cases is estimated to be 3.6% worldwide (71). Pyrazinamide, a first-line drug with remarkable sterilizing activity (39, 40, 79), has an important role in the treatment of both drug-susceptible and drug-resistant tuberculosis (74). MDR-TB with bacillary resistance to pyrazinamide would further worsen prognosis (43), and the rising prevalence of such MDR-TB is noted with concern (1). Thus, delineation of pyrazinamide resistance in isolates of MDR Mycobacterium tuberculosis has potential prognostic value. Standard culture-based pyrazinamide susceptibility tests are difficult to perform as a result of poor buffering of test media (79), use of acidic medium pH that inhibits growth (21, 41, 67), and excessively large inocula that reduce the activity of pyrazinamide (15, 41, 77), thereby leading to false resistance. Pyrazinamide resistance is largely caused by \textit{pncA} mutations (58). Alternative drug susceptibility testing (DST) methods based on the detection of \textit{pncA} mutations or pyrazinamidase activity may help determine susceptibility to pyrazinamide. Molecular tests indirectly applied on culture isolates can shorten the turnaround time of drug susceptibility testing to less than one day (46, 59, 68, 72). Initially developed to differentiate \textit{M. bovis} from \textit{M. tuberculosis} and \textit{M. avium} complex as well as \textit{M. marinum} from \textit{M. kansasii} (28), pyrazinamidase assays are relatively easy to perform and can often be done in parallel with culture-based drug susceptibility testing to independently measure the enzyme activity. A literature search through MEDLINE and EMBASE with key phrases did not show any previous systematic review on the diagnostic performance of DST methods based
on \textit{pncA} mutations or pyrazinamidase activity. This review aims at clarifying the role of these assays by evaluating their test performance in different clinical settings.

Materials and Methods

PubMed and OvidSP were used to search for biomedical articles from MEDLINE, life science journals, and EMBASE through 27 April 2011 for publications in English using the following key phrases containing key words in titles or abstracts with the help of Boolean operators (“and”, “or”): (1) pyrazinamidase; (2) \textit{pncA}; (3) susceptibility, susceptible, sensitiv*, or resistan*; and (4) tuberculosis. The asterisk denotes a wild card. The search algorithm used in PubMed is shown in an appendix in the online supplement.

Inclusion criteria

A study was included only in the presence of concurrent sensitivity and specificity with reference to standard phenotypic DST of \textit{M. tuberculosis} to pyrazinamide. Standard DST methods included agar or Lowenstein-Jensen proportion methods, a liquid medium method using Middlebrook 7H9 medium, BACTEC radiometric method, or BACTEC Mycobacterial Growth Indicator Tube 960 (MGIT). Data were extracted by the first author in duplicates.

Exclusion criteria
Data on sensitivity and specificity were grouped for meta-analysis by assay category and the nature of samples (culture isolates versus clinical specimens). A study or a set of data within a study would be excluded from meta-analysis in the presence of selection bias or when there were fewer than three sets of data in a group.

Test characteristics

Sensitivity refers to the proportion of pyrazinamide-resistant strains detected by the evaluated assay among pyrazinamide-resistant strains defined by the reference phenotypic assay. Specificity refers to the proportion of pyrazinamide-susceptible strains detected by the evaluated assay among pyrazinamide-susceptible strains defined by the reference phenotypic assay.

Summary estimates of sensitivity, specificity, positive likelihood ratio (LR) and negative LR were estimated by meta-analysis using a bivariate random effects model via SAS proc mixed procedure (53).

Meta-regression

Heterogeneity within each group of assays was explored by unweighted meta-regression analysis using the Moses-Shapiro-Littenberg method, which involves regression of the log diagnostic odds ratio against a measure of diagnostic threshold (25, 32, 48). Significant heterogeneity for a covariate was considered present with p-values $\leq 0.05$. Covariates included the reference DST method, whether the study cohort or sampling method was well-defined, whether the putative
regulatory region of \textit{pncA} was included, whether the Wayne method was considerably modified, and whether there could be considerable false resistance in the reference phenotypic assay. The last covariate was assumed to be present when > 10\% pyrazinamide-resistant strains had pyrazinamidase activity or no \textit{pncA} mutations without mention of retesting these strains.

Bivariate meta-analysis was repeated after excluding data sets with significant heterogeneity.

\textit{Funnel plot asymmetry}

Funnel plot asymmetry was examined by plotting the natural log diagnostic odds ratio against the reciprocal of the square root of effective sample size for each test method (14). Significant asymmetry was denoted by p-values \( \leq 0.05 \). Significant funnel plot asymmetry suggests either publication bias for studies with positive findings, or bias due to exaggerated estimates from smaller studies or studies of lower quality.

\textit{Prevalence of pyrazinamide resistance and predictive values}

Each included study was also examined for the respective prevalence of pyrazinamide resistance in samples of MDR and non-MDR \textit{M. tuberculosis} isolates. The critical prevalence levels required of each test method to attain predictive values \( \geq 85\% \) under different clinical settings were then calculated using the standard formulae: (1) pre-test odds = prevalence/ (1 - prevalence); (2) PPV = post-test odds/ (1 + post-test odds), where post-test odds = pre-test odds x positive LR; (3) NPV = 1 - post-test odds/ (1 + post-test odds), where post-test odds = pre-test odds x negative LR; (4) positive LR = sensitivity/ (1 - specificity); and (5) negative LR = (1 - sensitivity)/
specificity. PPV and NPV refer to positive and negative predictive values, respectively.

MetaDiSc version 1.4 (76), SAS Enterprise Guide 3.0, OpenOffice.org 3.0, and SPSS version 10 (Chicago, IL) were used for statistical analysis.

Results

Literature search initially identified 128 articles. Figure 1 shows how articles were selected for meta-analyses. All included data involved culture isolates of *M. tuberculosis*. Only one identified article contained concurrent data on sensitivity and specificity involving direct examination in specimens (62).

Tables S1 and S2 in the online supplement summarize major findings of 48 publications (1-4, 6, 8, 9, 11-13, 17, 18, 22-24, 26, 27, 29-31, 33-37, 42, 45-47, 49, 51, 52, 56-66, 68-70, 72, 75) included in the initial meta-analysis, which comprised 40 sets of data on molecular assays targeting *pncA* and 33 sets of data on pyrazinamidase assays using the Wayne method. All molecular assays covered the entire open reading frame of *pncA*. Except for two studies (13, 58), all mentioned sequencing upstream of *pncA*. Data for pyrazinamidase assays were inadequate for evaluating methods other than the Wayne method.

Meta-regression showed that the possible presence of considerable false resistance was the only major source of heterogeneity for both PCR-DNA sequencing (p-value = 0.006) and the Wayne
Table 1 shows results of the initial meta-analysis. Results of standard phenotypic DST were based on repeat findings, when validation of pyrazinamide-resistant isolates without \textit{pncA} mutations or with pyrazinamidase activity was made. Data pertaining to members of \textit{M. tuberculosis} complex other than \textit{M. tuberculosis} (such as \textit{M. bovis}, \textit{M. bovis} BCG, \textit{M. africanum}, and \textit{M. microti}) were excluded whenever possible. Molecular assays by line probe, branch migration inhibition, denaturing gradient gel electrophoresis, microarray, and temperature-mediated heteroduplex analysis using denaturing high-performance liquid chromatography were considered in one group. Compared with the Wayne assay, the summary estimate of sensitivity was non-significantly lower for PCR-DNA sequencing (89% vs. 87%, \(p = 0.60\)) whereas the summary estimate of specificity was significantly lower (97% vs. 93%, \(p = 0.04\)). The choice of reference culture-based drug susceptibility testing methods for evaluating test performance did not significantly change the summary estimates of sensitivity and specificity for evaluated assays. For PCR-DNA sequencing targeted at \textit{pncA}, summary estimates of sensitivity and specificity were 87% and 95%, 85% and 88%, and 90% and 94% when BACTEC radiometric method, MGIT, and Lowenstein Jensen or Middlebrook 7H10/11 proportion method were used as predominant reference assays, respectively. For the Wayne assay, summary estimates of sensitivity and specificity were 89% and 96%, 89% and 98%, 91% and 97%, and 86% and 97% when BACTEC radiometric method, MGIT, Lowenstein Jensen proportion method, and Middlebrook 7H10/11 proportion method were used as predominant reference assays, respectively. Funnel plot asymmetry was significant for both the Wayne assay (\(p\)-value=0.006), and PCR-DNA sequencing targeted at \textit{pncA} (\(p\)-value=0.04).
Table 2 shows results of the final meta-analysis restricted to studies that either showed no discrepancy between reference and evaluated assays, or retested pyrazinamide-resistant isolates in case of discrepancy. No meta-analysis was performed for PCR-SSCP as there were only two sets of data after applying the restriction. Summary estimates (95% confidence interval) of sensitivity and specificity were 92% (87%-95%) and 93% (88%-97%) for PCR-DNA sequencing, 98% (93%-100%) and 96% (87%-99%) for other molecular assays (line probe assay, microarray, branch migration inhibition, and denaturing gradient gel electrophoresis), and 91% (87%-94%) and 97% (94%-98%) for the Wayne assay. There was no significant difference in the summary estimates of sensitivity and specificity between PCR-DNA sequencing and the Wayne assay (p-values = 0.78 and 0.13, respectively). Funnel plot asymmetry was persistently significant (p-value = 0.03) for the Wayne assay, but non-significant for PCR-DNA sequencing (p-value = 0.07) and other molecular assays (p-value = 0.27).

The median prevalence of pyrazinamide resistance in culture isolates of MDR *M. tuberculosis* based on 14 studies included in the initial meta-analysis was 51% (range 31-89%) (1, 2, 4, 6, 9, 17, 24, 26, 42, 49, 59, 63, 69, 75). The median prevalence of pyrazinamide resistance in culture isolates of *M. tuberculosis* susceptible to both isoniazid and rifampicin based on eight studies was 5% (range 0%-9%) (6, 17, 24, 26, 34, 49, 59, 63).

Using summary estimates of sensitivity and specificity in the final meta-analysis for calculation, Table 3 shows the critical prevalence levels of pyrazinamide resistance required of three categories of pyrazinamide DST assays to attain different predictive values. Considering the
prevalence range of pyrazinamide resistance in different clinical settings, both molecular assays targeting \( pncA \) mutations and the Wayne assay can rule in pyrazinamide resistance in MDR \( M. \) \( tuberculosis \) isolates with PPV largely exceeding 90%, and rule out pyrazinamide resistance in non-MDR \( M \) \( tuberculosis \) isolates with NPV exceeding 99%.

Discussion

To our knowledge, this is the first systematic review and meta-analysis of molecular and pyrazinamidase assays for testing susceptibility of \( M. \) \( tuberculosis \) in culture isolates to pyrazinamide. Data were insufficient for evaluating these assays directly applied in clinical specimens, and insufficient for evaluating pyrazinamidase assays based on the McClatchy method (38) and the Russell’s phenol-hypochlorite method (5, 54). Assuming the prevalence of pyrazinamide resistance is 31%-89% in MDR \( M. \) \( tuberculosis \) culture isolates and 0%-9% in non-MDR isolates, this systematic review shows that molecular assays targeted at \( pncA \) and the putative regulatory region, especially PCR-DNA sequencing, may reliably detect pyrazinamide resistance in MDR \( M. \) \( tuberculosis \) strains and rule out pyrazinamide resistance in non-MDR stains. With significant funnel plot asymmetry in the final meta-analysis, the current review may have overestimated the actual test performance of the Wayne assay. Given the relatively few published data included in the current review, it remains to be confirmed whether other molecular assays, such as line probe assay, microarray, branch migration inhibition, and denaturing gradient gel electrophoresis, may attain higher predictive values than PCR-DNA sequencing.
Instead of estimating sensitivity and specificity separately by pooling in a univariate model without considering the threshold effect, or using the summary Receiver Operating Characteristic curve that removes the effect of a possible threshold by comparing diagnostic odds ratios rather than estimates of sensitivity and specificity, we have used a bivariate random effects model that takes into account the correlation between sensitivity and specificity within studies without masking important differences in these test performance estimates (25, 53, 73). The use of a random effects model allows for the presence of heterogeneity between studies.

As there may be concern about the performance of DST of M. tuberculosis to pyrazinamide in Middlebrook 7H10/11(47) and MGIT (10, 50), it is important to examine whether the choice of reference DST medium may affect the evaluation of test performance of molecular assays and pyrazinamidase assays. Among standard culture-based reference DST methods, BACTEC radiometric method is probably the most reliable (79) and currently the reference method of choice for pyrazinamide susceptibility testing (7), although its reliability has also been questioned (16). MGIT has widely replaced BACTEC radiometric method owing to the concern about disposal of radioactive substance. However, MGIT may over-report pyrazinamide resistance (10, 50), possibly because of several differences in the inoculum used in MGIT, in comparison with the BACTEC radiometric method (10). Lowenstein Jensen medium is considered acceptable provided that a good technique is used, whereas Middlebrook 7H10 is the least reliable (79). The bivariate random effects model used in the current review, which is capable of comparing sensitivity and specificity of different candidates, suggests no significant difference in the test performance between included studies that used BACTEC radiometric
The sensitivity of PCR-DNA sequencing targeted at \textit{pncA} may have been underestimated for two reasons. First, among 29 included studies on PCR-DNA sequencing targeted at \textit{pncA}, only nine mentioned retesting pyrazinamide-resistant isolates that contained no \textit{pncA} mutations (11, 18, 23, 24, 36, 47, 49, 57, 58). The lack of validation by retesting may leave false resistance unidentified, thereby reducing sensitivity. This might partly account for the relatively low sensitivity estimates in the range of 67\% to 85\% from nine studies included in the initial meta-analysis (3, 4, 8, 26, 30, 31, 66, 69, 75). The same problem might also apply to the Wayne assay; only 16 out of 33 included studies on the Wayne assay mentioned retesting pyrazinamide-resistant isolates that had pyrazinamidase activity, in the presence of discrepancy between reference and tested assays (2, 11-13, 24, 29, 36, 42, 45, 47, 49, 52, 57, 58, 61, 64). Second, the use of a relatively low resistance breakpoint in standard culture-based DST may also lead to misinterpretation of susceptible isolates as resistant (false resistance). Heifets has suggested that 300 mg/L (19-21) might be more appropriate than 100 mg/L (55) as the resistance breakpoint in the BACTEC radiometric method, while Zhang has suggested a compromised cutoff at 200 mg/L after estimating minimal inhibitory concentration (MIC) using the Henderson-Hasselbach equation (77, 79). Again, this problem may affect the Wayne assay.

The possible presence of false resistance in the reference assay was identified as the only major source of heterogeneity. Excluding data sets with possibly considerable false resistance resulted in an increase in the summary estimate (95\% confidence interval) of sensitivity from 87\%
(82%-91%) in the initial analysis to 92% (87%-95%) for PCR-DNA sequencing, and from 89%
(84%-92%) in the initial analysis to 91% (87%-94%) for the Wayne assay. Furthermore, funnel
plot asymmetry became non-significant for PCR-DNA sequencing in the final meta-analysis.

Although the current review suggests that the Wayne test might be sensitive and specific for
detecting pyrazinamide resistance, molecular assays are probably the way forward.

Notwithstanding the non-clustered distribution of \textit{pncA} mutations (57) and a relatively high cost
of molecular assays, recent advances in DNA sequencing technology will make molecular
detection of pyrazinamide resistance more affordable and rapid in the near future. The
requirement of a sufficient inoculum with considerable amount of bacilli for detecting
pyrazinamidase activity makes the Wayne assay prone to misinterpretation of some susceptible
isolates as resistant (false resistance) and incurs a delayed turnaround time.

A less expensive cost and a shorter turnaround time give \textit{pncA} sequencing an edge over the
BACTEC radiometric method based on MIC. Although alternative mechanisms of pyrazinamide
resistance such as unknown target alteration, uptake of pyrazinamide and efflux of pyrazinoic
acid may reduce the sensitivity of \textit{pncA} sequencing (45, 65, 78, 79), most pyrazinamide-resistant
strains, including those with low-level resistance (an increase in MIC by 2-3 folds), have \textit{pncA}
mutations (11, 57).

Besides the possibility of underestimating sensitivity of tested assays due to unidentified false
resistance in the reference assay, other limitations in the current review include the following.
First, the failure of the search algorithm in identifying all eligible studies as well as the exclusion
of publications in other languages from the current review could have introduced publication
bias. Second, heterogeneity exists between included studies (data not shown). Although the
random effects model allows for the presence of heterogeneity, there may still be some
controversy about combining study estimates in its presence. The estimate obtained from the
random effects model refers to a mean effect about which true study effects vary rather than a
universally true study effect that varies between studies due to sampling error. Third, although
restriction applied in the final meta-analyses may minimize underestimation of sensitivity by
reducing false resistance, technical problems inherent with standard culture-based pyrazinamide
susceptibility testing methods and the choice of breakpoint concentration may still lead to
misinterpretation of some susceptible isolates as resistant, thereby underestimating the sensitivity
of evaluated assays. Lastly, the current review has evaluated test performance with no reference
to clinical treatment outcomes or assurance of unique isolates by genotyping. This could have
introduced bias in validation.

In conclusion, in most epidemiological settings, PCR-DNA sequencing, and possibly other
molecular assays targeting at \textit{pncA} mutations, can reliably detect pyrazinamide resistance in
\textit{MDR} \textit{M. tuberculosis} isolates and rule out pyrazinamide resistance in non-MDR ones. Molecular
assays for detecting pyrazinamide resistance are probably the way forward.
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Transparency declarations
None to declare.
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   pyrazinamide and ofloxacin resistance among drug resistant Mycobacterium tuberculosis

   new mutations in pyrazinamide-resistant strains of Mycobacterium tuberculosis and
   identification of conserved regions important for the catalytic activity of the pyrazinamidase


53. **Reitsma, J. B., A. S. Glas, A. W. S. Rutjes, R. J. P. M. Scholten, P. M. Bossuyt, and A. H.**


Search in MEDLINE and EMBASE via PubMed and OVIDSP identified 128 articles in English

79 articles excluded for the following reasons:
- Irrelevant (n=49)
- No data on specificity (n=15)
- No standard phenotypic reference test (n=9)
- No data on sensitivity (n=5)
- Selection bias (n=1)

49 articles with 76 sets of data

Excluded because data < 3 sets: 1 article with 1 set of data on McClatchy method, 1 set of data on PCR-based micopyrazinamidase assay, 1 set of data on PCR-SSCP involving direct sputum specimens

Initial meta-analysis of 48 articles with 73 sets of data:
- PCR-DNA sequencing (29 sets), PCR-SSCP (5 sets), other molecular assays (6 sets)
- Wayne’s method (33 sets)

After meta-regression, which identified possibly considerable false resistance in the reference assay as the major source of heterogeneity, 9 articles with 11 sets of data, and another 8 sets of data were excluded. Consequently, one article (with one set of data) and another set of data on PCR-SSCP were further excluded as there were fewer than 3 sets of data in the test group.

Final meta-analysis of 38 articles with 52 sets of data:
- PCR-DNA sequencing (20 sets)
- Molecular assays other than PCR-DNA sequencing and PCR-SSCP (5 sets)
- Wayne’s method (27 sets)
Table 1 Initial meta-analyses of pyrazinamide susceptibility testing methods in culture isolates of *M. tuberculosis*

<table>
<thead>
<tr>
<th>Assay category</th>
<th>Sets of Resistant data strains</th>
<th>Susceptible strains</th>
<th>Mean (95% CI) sensitivity</th>
<th>Mean (95% CI) specificity</th>
<th>Mean positive LR (95% CI)</th>
<th>Mean negative LR (95% CI)</th>
<th>Mean DOR (95% CI)</th>
<th>Funnel plot (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-DNA</td>
<td>29</td>
<td>998</td>
<td>1207</td>
<td>87 (82-91)</td>
<td>93 (89-96)</td>
<td>12.9 (7.6-18)</td>
<td>0.14 (0.09-0.21)</td>
<td>93 (48-181)</td>
</tr>
<tr>
<td>PCR-SSCP with or without MPAC</td>
<td>5</td>
<td>228</td>
<td>261</td>
<td>75 (53-88)</td>
<td>95 (83-98)</td>
<td>14.1 (3.9-51)</td>
<td>0.27 (0.11-0.66)</td>
<td>53 (11-254)</td>
</tr>
<tr>
<td>Molecular assays comprising LiPA, microarray, BMI, DGGE, and TMHA-DHPLC</td>
<td>6</td>
<td>198</td>
<td>416</td>
<td>96 (89-99)</td>
<td>97 (89-99)</td>
<td>28.9 (8.3-100.8)</td>
<td>0.04 (0.01-0.12)</td>
<td>756 (138-4140)</td>
</tr>
<tr>
<td>The Wayne assay</td>
<td>33</td>
<td>1141</td>
<td>2052</td>
<td>89 (84-92)</td>
<td>97 (95-98)</td>
<td>27.8 (16.0-48.3)</td>
<td>0.12 (0.08-0.17)</td>
<td>237 (121-465)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, branch migration inhibition; CI, confidence interval; DGGE, denaturing gradient gel electrophoresis; DNA, deoxyribonucleic acid; DOR, diagnostic odds ratio; LiPA, line probe assay; LR, likelihood ratio; MPAC, multiplex PCR amplimer
conformation analysis; PCR, polymerase chain reaction; SSCP, single-stranded conformation polymorphism; TMHA-DHPLC, temperature-mediated heteroduplex analysis using denaturing high-performance liquid chromatography.

* Based on standard phenotypic drug susceptibility testing.
Table 2 Final meta-analyses excluding studies with possibly considerable false resistance in the reference assay

<table>
<thead>
<tr>
<th>Assay category</th>
<th>Sets of Resistant Strains</th>
<th>Sets of Susceptible Strains</th>
<th>Mean sensitivity (95% CI)</th>
<th>Mean specificity (95% CI)</th>
<th>Mean positive LR (95% CI)</th>
<th>Mean negative LR (95% CI)</th>
<th>Mean DOR (95% CI)</th>
<th>Funnel plot (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-DNA</td>
<td>20</td>
<td>715</td>
<td>92 (87-95)</td>
<td>93 (88-97)</td>
<td>13.8 (7.0-27.3)</td>
<td>0.09 (0.05-0.14)</td>
<td>158</td>
<td>0.07</td>
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<tr>
<td>sequencing</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Molecular assays</td>
<td>5</td>
<td>185</td>
<td>98 (93-100)</td>
<td>96 (87-99)</td>
<td>27.7 (6.6-116.4)</td>
<td>0.02 (0.01-0.07)</td>
<td>1438 (202-10230)</td>
<td>0.27</td>
</tr>
<tr>
<td>comprising LiPA, microarray, BMI, and DGGE</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>The Wayne assay</td>
<td>27</td>
<td>912</td>
<td>91 (87-94)</td>
<td>97 (94-98)</td>
<td>27.3 (14.4-51.7)</td>
<td>0.09 (0.06-0.14)</td>
<td>296 (138-635)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, branch migration inhibition; CI, confidence interval; DGGE, denaturing gradient gel electrophoresis; DNA, deoxyribonucleic acid; DOR, diagnostic odds ratio; LiPA, line probe assay; LR, likelihood ratio; PCR, polymerase chain reaction.

* Based on standard phenotypic drug susceptibility testing.
Table 3  Prevalence levels of pyrazinamide resistance in culture isolates of *M. tuberculosis* for attaining different predictive values

<table>
<thead>
<tr>
<th>Pyrazinamide susceptibility testing assay</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥ 85%</td>
<td>≥ 90%</td>
</tr>
<tr>
<td>PCR-DNA sequencing targeted at pncA mutations</td>
<td>≥ 29%</td>
<td>≥ 39%</td>
</tr>
<tr>
<td>Other molecular assays targeting pncA mutations</td>
<td>≥ 17%</td>
<td>≥ 25%</td>
</tr>
<tr>
<td>The Wayne assay</td>
<td>≥ 17%</td>
<td>≥ 25%</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, branch migration inhibition; CI, confidence interval; DGGE, denaturing gradient gel electrophoresis; DNA, deoxyribonucleic acid; DOR, diagnostic odds ratio; LiPA, line probe assay; LR, likelihood ratio; NPV, negative predictive values; PCR, polymerase chain reaction; PPV, positive predictive value.

* Based on estimates of sensitivity and specificity obtained in the final meta-analysis.