Real-time PCR assay for Mycobacterium tuberculosis: a systematic review and meta-analysis

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Abstract:

Background: Numerous studies on in-house real-time PCR (hRT-PCR) assay for the detection of Mycobacterium tuberculosis (Mtb) have been reported. To assess the overall accuracy of the test in diagnosing pulmonary and extra-pulmonary Mtb infection, a systematic review and meta-analysis was performed.

Methods: Various databases like PUBMED, EMBASE, Web of Science, and Cochrane were searched for studies that estimated diagnostic sensitivity and specificity with the hRT-PCR assay in samples from pulmonary and extra-pulmonary Mtb infected patients, with Mtb culture as the reference standard. Bivariate random effect models were used to provide pooled estimation of diagnostic accuracy. Further, subgroup and meta-regression analyses were performed to explore sources of heterogeneity. The risk of bias was assessed by the QUADAS-2 tool.

Results: Out of the 3589 studies, 18 studies met the inclusion criteria., The pooled sensitivity and specificity of hRT-PCR assay were 0.96 and 0.92, respectively when compared to Mtb culture data. The diagnostic odds ratio (DOR) was 192.96 (95% CI 68.46, 543.90), and the area under the summary ROC curve (AUC) was 0.9791. There was significant heterogeneity in sensitivity and specificity among the enrolled studies ($p < 0.001$). The studies with high-quality assessment and involving respiratory specimen were associated with better accuracy.

Conclusions: The results suggest the usefulness of hRT-PCR assay for the diagnosis of TB with high sensitivity and specificity in low-income/high-burden settings

Keywords: Tuberculosis, Laboratory diagnosis, In-house real-time PCR, Meta-analysis, Systematic review

Introduction:

Tuberculosis (TB) continues to be a worldwide major public health problem. As per the Global TB Report 2021, the estimated incidence of all forms of TB in India for the year 2020 was 188 per 100,000 population (129-257 per 100,000 population). A total of 19,33,381 incident TB patients (new & relapse) were notified during 2021 which was 19% higher than that of 2020 (16,28,161)[1.] Hence rapid easier diagnosis and treatment are pivotal factors for the effective control of TB[2.] Acid-fast staining and Mtb culture are classical Mtb diagnostic tests. The acid-fast stain lacks sensitivity, and the culture which is the gold standard test requires several weeks for incubation[3,4.] These kind of limitations of the standard available tests lead to exploring newer options for early rapid laboratory diagnosis[5.] Nucleic acid amplification tests (NAATs), are being used for TB diagnosis, but it carries the disadvantage of false positives[6.] Some commercial tests like COBAS TaqMan, Xpert MTB/RIF and the Abbott Real-Time MTB assay, are being used for TB diagnosis in recent times[7–9.] However, many clinical laboratories in suburban areas with high TB burden cannot afford these assays due to limitations in infrastructure and resources[1.] In-house polymerase chain reaction (hPCR) may be more affordable, feasible, and sustainable than Xpert MTB/RIF in resource limited settings[10.] Several regions like IS6110 and 16S rDNA, have been used as targets for Mtb assays[11–13.] PCR technologies have improved markedly with the development of RT-PCR for the detection of Mtb infection, having an edge over conventional PCR in speed, automation, high sensitivity and specificity, and a low risk of cross-contamination[17,18, 19.] Inhouse RT-PCR would be particularly popular in resource limited countries like Brazil, India, China, the Russian Federation, Southeast Asian, South Africa, and East Africa. Although recent studies have revealed that RT-PCR assays have good diagnostic performance for TB, there are discrepancies between their results[10,20–32.] Therefore, by systematic review and meta-analysis, we explored factors associated with heterogeneity as well as diagnostic accuracy of the hRT-PCR assay for TB using data from previous studies

Methods:

The current meta-analysis was conducted according to the PRISMA guidelines[33.] Since the study was a systematic review and meta-analysis of published articles, patient consent or approval from the institutional ethics committee was not necessary.

Search strategy: Databases like PUBMED, EMBASE, Web of Science and Cochrane Library were searched for "tuberculosis", "Mycobacterium tuberculosis", "nucleic acid amplification techniques", "real time PCR", "quantitative real-time polymerase chain reaction", "PCR, quantitative real-time", "quantitative realtime PCR", "real-time PCR, quantitative", "sensitivity and specificity", or "predictive value". In addition, the references of reviews on NAATs were searched for possible articles.

Study selection: All available studies that reported the assessment of hRT-PCR assay for detection of TB were included in the analysis. The following type of studies were excluded (i) the reference standard was not culture proven Mtb; (ii) studies performed with other assays other than hRT-PCR assay; (iii) application of hRT- PCR assay for determining drug resistance; (iv) incomplete; (v) evaluation of hRTPCR assay on animal specimens; and (vi) conference abstracts, letters, case reports, editorials, and reviews without original data were excluded. We screened the literature by looking up the title and abstract. Then, the full texts were carefully read to determine whether they could be included.

Data extraction and quality assessment: We extracted accurate information from the uincluded articles. The quality of the included studies was assessed using a Revised Tool for the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2),

which consists of seven domains[34]. A study with no domain of a high risk of bias and no domain of high applicability concerns were determined to be of high quality.

Statistical analysis: Meta-Disc (version 1.4) software was used for analysis[35]. DerSimonian-Laird random effects model (REM) was used for pooling with the following estimates: sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), and the diagnostic odds ratio (DOR). A summary receiver operating characteristic (SROC) curve was also constructed for the hRT-PCR assay[36]. A shoulder-like curve illustrates that the variability between studies may be due to the threshold effect. A non-shoulder-like curve indicates that sensitivity and specificity are not correlated. The overall diagnostic performance of that hRT-PCR assay was assessed as the area under the curve (AUC) (an AUC value of 100% indicates a perfect test, while an AUC of 50% signifies poor diagnostic accuracy)[37, 38]. Chi-square and Fisher's exact tests were used to detect statistically significant heterogeneity. Heterogeneity between included studies was evaluated with subgroup (stratified) analysis and meta-regression analysis[39]. In the subgroup analysis, we computed pooled DOR estimates in various strata. The following factors as potential sources of heterogeneity: study design, target sequence, respiratory specimen versus non-respiratory specimen, the distribution of TB, and components of study quality. The meta-regression model produces relative diagnostic odds ratios (RDOR) as the output[39]. An RDOR of 1.0 explains that a particular covariate does not affect the overall DOR. An RDOR> 1.0 explains that studies with a particular characteristic have a higher DOR than studies without this characteristic. For an RDOR< 1.0, the converse is true. The potential publication bias of included studies was assessed by Deeks's funnel plot (Stata version 12.0; Stata Corp., College Station, TX) [40].

Results

Study search: Out of the 3589 articles, we identified 15 eligible articles representing 18 independent studies (Fig. 1). The performance of the hRT-PCR assay in Mtb detection of clinical specimens was evaluated from all included studies with Mtb culture as a reference standard. Summary characteristics of the included studies are shown in Table 1. Eleven studies used respiratory specimens, and five used non-respiratory specimens. Two studies focused on patients with HIV-associated TB. Five studies were from Brazil, two were from India, and the remaining studies were from eight different countries. Among them, eight are the high TB burden countries. Eleven studies used IS6110 as an amplification target, and 7 studies used other targets (e.g., MPT64 and SenX3- RegX3). A total of 3281 samples, including 2809 respiratory samples and 472 non-respiratory samples were used of results calculation.

Quality evaluation: QUADAS2 was used for quality assessment. Three studies were deemed to be of case-control design, which compared diagnosed TB patients to non-TB individuals, hence had a risk of selection bias. Four studies failed to illustrate the blind working flow, hence failed to pass "index test" evaluation. Two studies did not provide sufficient description concerning the reference test results. No other domain had a high risk of bias or a high applicability concern (Fig. 2).

Diagnostic accuracy of hRT-PCR assay: When all 18 studies using the hRT-PCR assay were evaluated together, the overall sensitivity and specificity estimates were 0.96 (95% CI 0.95, 0.96) and 0.92 (95% CI 0.90, 0.93), respectively. The sensitivity and specificity of all studies are shown in the forest plot (Fig. 3a, b). The overall LR+ was 16.90 (95% CI 7.22, 39.56), and LR- was 0.11 (95% CI 0.06, 0.18). The pooled DOR was 192.96 (95% CI 68.46, 543.90). Heterogeneity was detected by chi-square analysis in the summary results. All measurements showed high heterogeneity ($p < 0.001$ for the test of heterogeneity). The accuracy was measured, and their corresponding chi-square test was applied to statistically analyse heterogeneity (Table 2). The overall accuracy of the hRT-PCR assay in a summary receiver operating characteristic (SROC) curve is displayed in Fig. 4, and the curve displayed a trade-off between sensitivity and specificity. The area under the SROC curve (AUC) was 0.9791, indicating a highly diagnostic accuracy. Overall, significant heterogeneity in sensitivity and specificity was observed in the clinical applications of the hRT-PCR assay in TB detection.

Exploration of heterogeneity: The threshold effect, method differences and study characteristics may lead to the variability. The SROC curve with studies was weighted by their inverse variance, as shown in Fig. 4. The non-shoulder-like curve indicated no threshold effect in the current metaanalysis. Furthermore, the Spearman correlation coefficient was 0.147, and the p value was 0.562. It illustrated no threshold effect. Subgroup analysis was also used to explore other factors that were associated with heterogeneity by stratifying data into relatively more homogeneous strata. The DOR estimates of the study characteristics are compared in Table 3. The heterogeneity could be explained in some strata, including specimen type, the distribution of TB, and quality of studies. However, even after stratification, the heterogeneity remained in the evaluation of diagnostic accuracy. We further performed a meta-regression analysis to explain the variation after subgroup analysis. As shown in Table 4, the RDOR was established from the meta-regression analysis using the restricted maximum likelihood (REML) method to measure between-study variance. Studies with respiratory specimens produced RDOR values that were significantly higher than those used non-respiratory specimens or both specimens. Studies with a high-quality level produced RDOR that were significantly higher than those with medium quality levels or low-quality levels. The distribution of TB displayed a slightly higher RDOR but no statistical significance in the final regression model. Study design and target sequence did not produce a significant RDOR, indicating that the use of any study design and target sequence did not substantially affect diagnostic accuracy. Therefore, specimen types and quality of studies may affect accuracy heterogeneity. Evaluation of the Deeks' ($p = 0.11$) test did not show evidence of publication bias. Furthermore, the funnel plot did not display the presence of asymmetry (Fig. 5).

Discussion

Principal findings: The meta-analysis was performed on 18 independent studies with a total of 97% AUC, indicating that the hRT-PCR assay for TB detection was useful in rapidly identifying TB cases and that negative data guaranteed the certainty for ruling out active TB. Since there is significant heterogeneity, subgroup and metaregression analysis indicated that the use of respiratory specimens and studies with high quality were associated with better diagnostic accuracy of hRT-PCR.

Clinical implications: Even though the power in evaluating the overall diagnostic accuracy of hRT-PCR is significant, determination of clinical accuracy is difficult due to significant heterogeneity. Our results showed that respiratory specimens and high-quality design were associated with better diagnostic accuracy of the hRT-PCR assay, which was consistent with a recent meta-analysis of the Xpert MTB/RIF PCR assay for the diagnosis of extra-pulmonary TB. There was a performance difference in the specimen site, with low sensitivity in pleural fluid (37%) and cerebrospinal fluid samples (69%), given the paucibacillary nature of these specimen[42.] Some researchers were concerned that the case-control study might overestimate the diagnostic accuracy since it samples patients from the extreme ends of the clinical spectrum (an ideal, "extreme contrast" setting). For example, the sensitivity of a test is evaluated in seriously diseased subjects, and the specificity in healthy individuals[43-46.] In our meta-analyses, laboratory factors (such as target sequence and amplification technique) weighted more on accuracy than study design features. The IS6110 gene was widely used for both pulmonary and extra-pulmonary TB diagnosis[13, 47, 48.] Due to its multiple copies in the genome of the Mtb complex, PCR might result in better sensitivity[14.] However, our data demonstrated that study design with IS6110 had little impact on diagnostic accuracy. This is possible because RT-PCR used in our enrolled studies carries better advanced technology compared to conventional PCR. RT-PCR uses built-in automated thermocyclers and fluorimeters to monitor PCR reactions in a single tube format in which the reaction processes rapidly and minimizes the risk of contamination from product carryover[49.] Therefore, RT-PCR can provide reliable and repeatable results. The performance of hRT-PCR was heterogeneous across studies; some patients could have false-positive hRT-PCR results and others false negative. Accuracy is related to the standard/reference assay, TB culture. Reliability is based on clinical diagnosis of TB disease. However, not all recruited studies have evaluated their hRTPCR according to these standards. Caution is highly necessary for the clinical implications and applicability of hRT-PCR. The combination with other clinical information, such as the disease history, family medical records, microscopy screening and histopathology data, is recommended in clinical practice.

Limitations of the review: Only one study evaluated the diagnostic test accuracy of the hRT-PCR assay for smear status, and only two studies included HIV-positive patients. Therefore, we could not determine the effect of smear and HIV status on the accuracy of the hRT-PCR assay. Second, we only included published studies in English, and this could have caused bias in our conclusion. Third, despite the fact that the subgroup analysis and meta-regression analysis could explain part of the observed heterogeneity in accuracy estimates, considerable heterogeneity remained unexplained. Finally, although we searched as many sources as possible, some eligible studies may have been missed.

Conclusion:

In conclusion, based on the meta-analysis using the bivariate model, the diagnostic accuracy of the hRT-PCR assay for TB detection was acceptable. Subgroup and meta-regression analyses were performed, and we found that the diagnostic characteristics were different, depending on the specimen type and quality of the studies. Thus, the hRT-PCR assay, a relatively inexpensive assay compared to other commercial kits, has potential practical value for diagnosing TB, especially in low-income/ high-burden settings, where infrastructures and medical resources are limited.

References:

1. WHO: Global tuberculosis report 2021.
2. Tiwari RP, Hattikudur NS, Bharmal RN, Kartikeyan S, Deshmukh NM, Bisen PS. Modern approaches to a rapid diagnosis of tuberculosis: promises and challenges ahead. Tuberculosis (Edinb). 2007;87(3):193–201.
3. Chagas M, Silva RM, Bazzo ML, Santos JI. The use of polymerase chain reaction for early diagnosis of tuberculosis in mycobacterium tuberculosis culture. Braz J Med Biol Res. 2010;43(6):543–8.
4. Shinu P, Nair A, Singh V, Kumar S, Bareja R. Evaluation of rapid techniques for the detection of mycobacteria in sputum with scanty bacilli or clinically evident, smear negative cases of pulmonary and extra-pulmonary tuberculosis. Mem Inst Oswaldo Cruz. 2011;106(5):620–4.
5. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, Kop J, Owens MR, Rodgers R, Banada P, et al. Rapid detection of mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol. 2010;48(1):229–37.
6. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. Am J Respir Crit Care Med. 2000;161(4 Pt 1):1376–95. https://doi.org/10.1164/ ajrccm.161.4.16141.
7. Horita N, Yamamoto M, Sato T, Tsukahara T, Nagakura H, Tashiro K, Shibata Y, Watanabe H, Nagai K, Nakashima K, et al. Sensitivity and specificity of Cobas TaqMan MTB real-time polymerase chain reaction for culture-proven mycobacterium tuberculosis: meta-analysis of 26999 specimens from 17 studies. Sci Rep. 2015;5:18113.
8. Morales-Lopez SE, Yepes JA, Anzola I, Aponte H, Llerena-Polo CR. Paradoxical results of two automated real-time PCR assays in the diagnosis of pleural tuberculosis. Int J Infect Dis. 2017;54:36–8.
9. Park KS, Kim JY, Lee JW, Hwang YY, Jeon K, Koh WJ, Ki CS, Lee NY. Comparison of the Xpert MTB/RIF and Cobas TaqMan MTB assays for detection of mycobacterium tuberculosis in respiratory specimens. J Clin Microbiol. 2013;51(10):3225–7.
10. Barletta F, Vandelannoote K, Collantes J, Evans CA, Arevalo J, Rigouts L. Standardization of a TaqMan-based real-time PCR for the detection of mycobacterium tuberculosis-complex in human sputum. Am J Trop Med Hyg. 2014;91(4):709–14.

11. Kox LF, van Leeuwen J, Knijper S, Jansen HM, Kolk AH. PCR assay based on DNA coding for 16S rRNA for detection and identification of mycobacteria in clinical samples. J Clin Microbiol. 1995;33(12):3225–33.
12. Lee BW, Tan JA, Wong SC, Tan CB, Yap HK, Low PS, Chia JN, Tay JS. DNA amplification by the polymerase chain reaction for the rapid diagnosis of tuberculous meningitis. Comparison of protocols involving three mycobacterial DNA sequences, IS6110, 65 kDa antigen, and MPB64. J Neurol Sci. 1994;123(1–2):173–9.
13. Thierry D, Brisson-Noel A, Vincent-Levy-Frebault V, Nguyen S, Guesdon JL, Gicquel B. Characterization of a mycobacterium tuberculosis insertion sequence, IS6110, and its application in diagnosis. J Clin Microbiol. 1990; 28(12):2668–73.
14. Miller N, Cleary T, Kraus G, Young AK, Spruill G, Hnatyszyn HJ. Rapid and specific detection of mycobacterium tuberculosis from acid-fast bacillus smearpositive respiratory specimens and BacT/ALERT MP culture bottles by using fluorogenic probes and real-time PCR. J Clin Microbiol. 2002;40(11):4143–7.
15. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, Yao JD, Wengenack NL, Rosenblatt JE, Cockerill FR 3rd, et al. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clin Microbiol Rev. 2006;19(1):165–256.
16. Palomino JC. Nonconventional and new methods in the diagnosis of tuberculosis: feasibility and applicability in the field. Eur Respir J. 2005;26(2): 339–50.
17. Aggarwal M, Aggarwal KC, Nair D. Have we made any headway in the diagnosis of Neurotuberculosis? Place of real time PCR. J Pediatr Infect Dis. 2012;07(04):139–43.
18. Albuquerque YM, Lima AL, Lins AK, Magalhaes M, Magalhaes V. Quantitative real-time PCR (q-PCR) for sputum smear diagnosis of pulmonary tuberculosis among people with HIV/AIDS. Rev Inst Med Trop Sao Paulo. 2014;56(2):139–42.
19. Chaidir L, Ganiem AR, Vander Zanden A, Muhsinin S, Kusumaningrum T, Kusumadewi I, van der Ven A, Alisjahbana B, Parwati I, van Crevel R. Comparison of real time IS6110-PCR, microscopy, and culture for diagnosis of tuberculous meningitis in a cohort of adult patients in Indonesia. PLoS One. 2012;7(12):e52001.
20. Darban-Sarokhalil D, Imani Fooladi AA, Maleknejad P, Bameri Z, Aflaki M, Nomanpour B, Yaslianifard S, Modarresi MH, Feizabadi MM. Comparison of smear microscopy, culture, and real-time PCR for quantitative detection of mycobacterium tuberculosis in clinical respiratory specimens. Scand J Infect Dis. 2013;45(4):250–5.
21. Gallo JF, Pinhata JM, Chimara E, Goncalves MG, Fukasawa LO, Oliveira RS. Performance of an in-house real-time polymerase chain reaction for identification of mycobacterium tuberculosis isolates in laboratory routine diagnosis from a high burden setting. Mem Inst Oswaldo Cruz. 2016;111(9):545–50.
22. Inoue M, Tang WY, Wee SY, Barkham T. Audit and improve! Evaluation of a real-time probe-based PCR assay with internal control for the direct detection of mycobacterium tuberculosis complex. Eur J Clin Microbiol Infect Dis. 2011;30(1):131–5.
23. Lee HS, Park KU, Park JO, Chang HE, Song J, Choe G. Rapid, sensitive, and specific detection of mycobacterium tuberculosis complex by real-time PCR on paraffin-embedded human tissues. J Mol Diagn. 2011;13(4):390–4.
24. Lira LA, Santos FC, Carvalho MS, Montenegro RA, Lima JF, Schindler HC, Montenegro LM. Evaluation of a IS6110-Taqman real-time PCR assay to detect mycobacterium tuberculosis in sputum samples of patients with pulmonary TB. J Appl Microbiol. 2013;114(4):1103–8.
25. Lyra JM, Maruza M, Verza M, Carneiro MM, Albuquerque Mde F, Rossetti ML, Ximenes R, Braga MC, Lucena-Silva N. Evaluation of four molecular methods for the diagnosis of tuberculosis in pulmonary and blood samples from immunocompromised patients. Mem Inst Oswaldo Cruz. 2014;109(6):805–13.
26. Miller MB, Popowitch EB, Backlund MG, Ager EP. Performance of Xpert MTB/ RIF RUO assay and IS6110 real-time PCR for mycobacterium tuberculosis detection in clinical samples. J Clin Microbiol. 2011;49(10):3458–62.
27. Rao P, Chawla K, Shenoy VP, Mukhopadhyay C. Role of real-time PCR for detection of tuberculosis and drug resistance directly from clinical samples. Indian J Tuberc. 2016;63(3):149–53.
28. Rozales FP, Machado AB, De Paris F, Zavascki AP, Barth AL. PCR to detect mycobacterium tuberculosis in respiratory tract samples: evaluation of clinical data. Epidemiol Infect. 2014;142(7):1517–23.
29. Sanjuan-Jimenez R, Toro-Peinado I, Bermudez P, Colmenero JD, Morata P. Comparative study of a real-time PCR assay targeting senX3-regX3 versus other molecular strategies commonly used in the diagnosis of tuberculosis. PLoS One. 2015;10(11):e0143025.
30. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg. 2010;8(5):336–41.
31. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011; 155(8):529–36.
32. .Zamora J, Abraira V, Muriel A, Khan K, Coomarasamy A. Meta-DiSc: a software for meta-analysis of test accuracy data. BMC Med Res Methodol. 2006;6:31.
33. Littenberg B, Moses LE. Estimating diagnostic accuracy from multiple conflicting reports: a new meta-analytic method. Med Decis Making. 1993; 13(4):313–21.
34. Deeks JJ. Systematic reviews in health care: systematic reviews of evaluations of diagnostic and screening tests. BMJ. 2001;323(7305):157–62.
35. Irwig L, Macaskill P, Glasziou P, Fahey M. Meta-analytic methods for diagnostic test accuracy. J Clin Epidemiol. 1995;48(1):119–30 discussion 131-112.

36. Lijmer JG, Bossuyt PM, Heisterkamp SH. Exploring sources of heterogeneity in systematic reviews of diagnostic tests. Stat Med. 2002;21(11):1525–37.
37. Song F, Khan KS, Dinnes J, Sutton AJ. Asymmetric funnel plots and publication bias in meta-analyses of diagnostic accuracy. Int J Epidemiol. 2002;31(1):88–95.
38. Flores LL, Pai M, Colford JM Jr, Riley LW. In-house nucleic acid amplification tests for the detection of mycobacterium tuberculosis in sputum specimens: meta-analysis and meta-regression. BMC Microbiol. 2005;5:55.
39. Penz E, Boffa J, Roberts DJ, Fisher D, Cooper R, Ronksley PE, James MT. Diagnostic accuracy of the Xpert(R) MTB/RIF assay for extra-pulmonary tuberculosis: a meta-analysis. Int J Tuberc Lung Dis. 2015;19(3):278–84 i-iii.
40. Kocagoz T, Yilmaz E, Ozkara S, Kocagoz S, Hayran M, Sachedeva M, Chambers HF. Detection of mycobacterium tuberculosis in sputum samples by polymerase chain reaction using a simplified procedure. J Clin Microbiol. 1993;31(6):1435–8.
41. Nolte FS, Metchock B, McGowan JE Jr, Edwards A, Okwumabua O, Thurmond C, Mitchell PS, Plikaytis B, Shinnick T. Direct detection of mycobacterium tuberculosis in sputum by polymerase chain reaction and DNA hybridization. J Clin Microbiol. 1993;31(7):1777–82.
42. Noordhoek GT, Kaan JA, Mulder S, Wilke H, Kolk AH. Routine application of the polymerase chain reaction for detection of mycobacterium tuberculosis in clinical samples. J Clin Pathol. 1995;48(9):810–4.
43. Knottnerus JA, van Weel C, Muris JW. Evaluation of diagnostic procedures. BMJ. 2002;324(7335):477–80.
44. Negi SS, Anand R, Pasha ST, Gupta S, Basir SF, Khare S, Lal S. Diagnostic potential of IS6110, 38kDa, 65kDa and 85B sequence-based polymerase chain reaction in the diagnosis of mycobacterium tuberculosis in clinical samples. Indian J Med Microbiol. 2007;25(1):43.
45. van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, Hermans P, Martin C, McAdam R, Shinnick TM, et al. Strain identification of mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol. 1993;31(2):406–9.
46. Cockerill FR 3rd. Application of rapid-cycle real-time polymerase chain reaction for diagnostic testing in the clinical microbiology laboratory. Arch Pathol Lab Med. 2003;127(9):1112–20.
47. Ling DI, Flores LL, Riley LW, Pai M. Commercial nucleic-acid amplification tests for diagnosis of pulmonary tuberculosis in respiratory specimens: meta-analysis and meta-regression. PLoS One. 2008;3(2):e1536.
48. Organization WHO. Tuberculosis diagnostic Xpert MTB/RIF test. Geneva: WHO; 2014.
49. Detjen AK, DiNardo AR, Leyden J, Steingart KR, Menzies D, Schiller I, Dendukuri N, Mandalakas AM. Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. Lancet Respir Med. 2015;3(6):451–61.
50. Yu L, Xu Y, Wang F, Yang C, Liu G, Song X. Functional roles of pattern recognition receptors that recognize virus nucleic acids in human adiposederived mesenchymal stem cells. Biomed Res Int. 2016;2016:9872138.
51. Nagai K, Horita N, Yamamoto M, Tsukahara T, Nagakura H, Tashiro K, Shibata Y, Watanabe H, Nakashima K, Ushio R, et al. Diagnostic test accuracy of loop-mediated isothermal amplification assay for mycobacterium tuberculosis: systematic review and meta-analysis. Sci Rep. 2016;6:39090

Annexures:

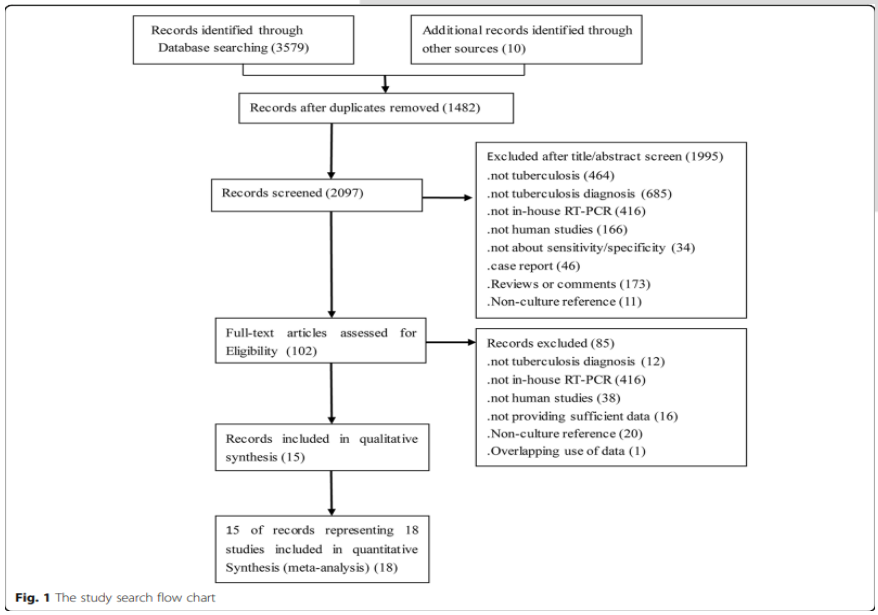

Fig. 1 The study search flow chart

Table 1 Characteristics of the included studies

Author Year	Study design	Country	Number of participants	Number of samples sent for culture	Culture+/−	Type of test (target sequence)	Acid-fast stain	Culture	Respiratory specimen	Non-respiratory specimen	R/NR	TP	FP	FN	TN
Aggarwal 2012 [20]	Cross-sectional	India	80	80	18/62	16 s rRNA	?	MGIT	–	csf	0/80	12	14	6	48
Albuquerque 2014 [21]	Cross-sectional	Brazil	140	140	47/93	IS6110	ZN	7H9,LJ	sp	–	140/0	41	1	6	92
Barletta 2014 [10]	Cross-sectional	Peru	112	112	84/28	IS6110	ZN	LJ	sp	–	109/0	79	1	5	25
Chaidir 2013 [22]	Case-control	Indonesia	230	230	102/105	IS6110	ZN	Liquid and solid	–	csf	0/207	94	46	8	59
Darban-Sarokhalil 2012 [23]	Case-control	Iran	247	247	112/135	cyp141	ZN	LJ	sp	–	247/0	101	3	11	132
Gallo 2016 [24]	Cross-sectional	Brazil	?	1451	1351/100	mpt64	?	Liquid and solid	sp	–	1451/0	1347	4	4	96
Inoue 2011 [25]	Cross-sectional	Singapore	414	414	55/128	IS6110	?	MGIT	sp	csf,pf,ti	104/66	43	3	8	116
Lee 2011 [26]	Case-control	Korea	370	129	53/76	senX3-regX3	ZN	3% Ogawa	ti	ti	53/76	47	1	16	65
Lira, LA 2012 [27]	Case-control	Brazil	165	165	66/99	IS6110	ZN	LJ	sp	–	165/0	58	2	8	97
Lyra 2014 [28]	Cross-sectional	Brazil	181	194	11/91	IS6110	ZN	LJ	sp	–	102/0	11	3	0	88
Miller 2011 [29]	Cross-sectional	America	90	112	89/23	IS6110	?	MGIT, LJ,7H11	sp,ba,bal.,ti	ln,ab,pf,ti	89/23	30	4	7	71
Rao 2016 [30]	Cross-sectional	India	100	200	44/56	16sRNA	?	MGIT	sp	–	100/0	44	2	0	54
Rozales 2014 [31]	Cross-sectional	Brazil	447	447	42/405	IS6110	ZN	7H9,MGIT	sp,bal	–	124/0	41	7	1	75
Sanjuan-Jimenez 2015 [32]	Case-control	Spain	153	145	76/69	senX3-regX3	ZN	LJ,MGIT	sp,ba,bal	pf,ln,ur,csf,ar	125/20	65	0	11	69
Sanjuan-Jimenez 2015 [32]	Case-control	Spain	153	145	76/69	IS6110	ZN	LJ,MGIT	sp,ba,bal	pf,ln,ur,csf,ar	125/20	72	9	4	60

Acid-fast stain: ZN, Ziehl-Neelsen; Culture: MGIT, Mycobacteria growth indicator tube; LJ, Löwenstein-Jensen; 7H9, Middlebrook 7H9 Broth. Respiratory specimen: sp., sputum; ba, broncheal/tracheal aspirate; bal, bronchialalveolar lavage; ti, tissue specimen. Non-respiratory specimen: ln, lymph node; pf, pleural fluid; ar, articular fluid; ab, abcess/pus; ur, urine; csf, cerebrospinal fluid; ti, tissue sample. Specimen number: R, number of the respiratory specimens; NR, number of the non-respiratory specimens. "?" represents that the specific method is not mentioned in this paper. TP, true-positive; FP, false-positive; FN, false-negative; TN, true-negative

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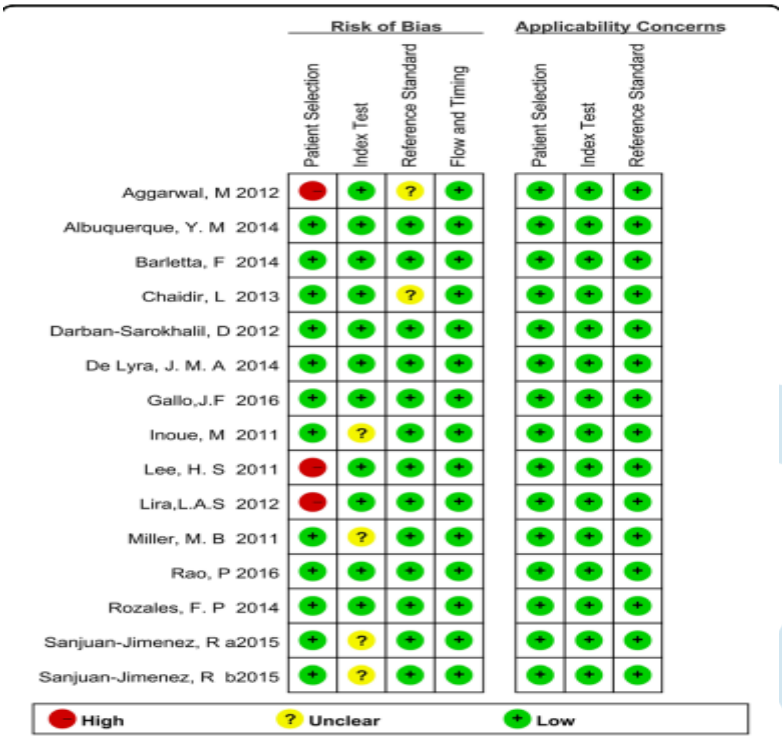

Fig. 2 Summary of methodological quality of studies according to the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies-2) tool. High-quality study: a study that had no domain with a high risk of bias and no domain with high applicability concerns; medium/moderate-quality study: a study that had domain with an unclear risk of bias or domain with unclear applicability concerns; low-quality study: a study that had a domain with a high risk of bias and domain with high applicability concerns

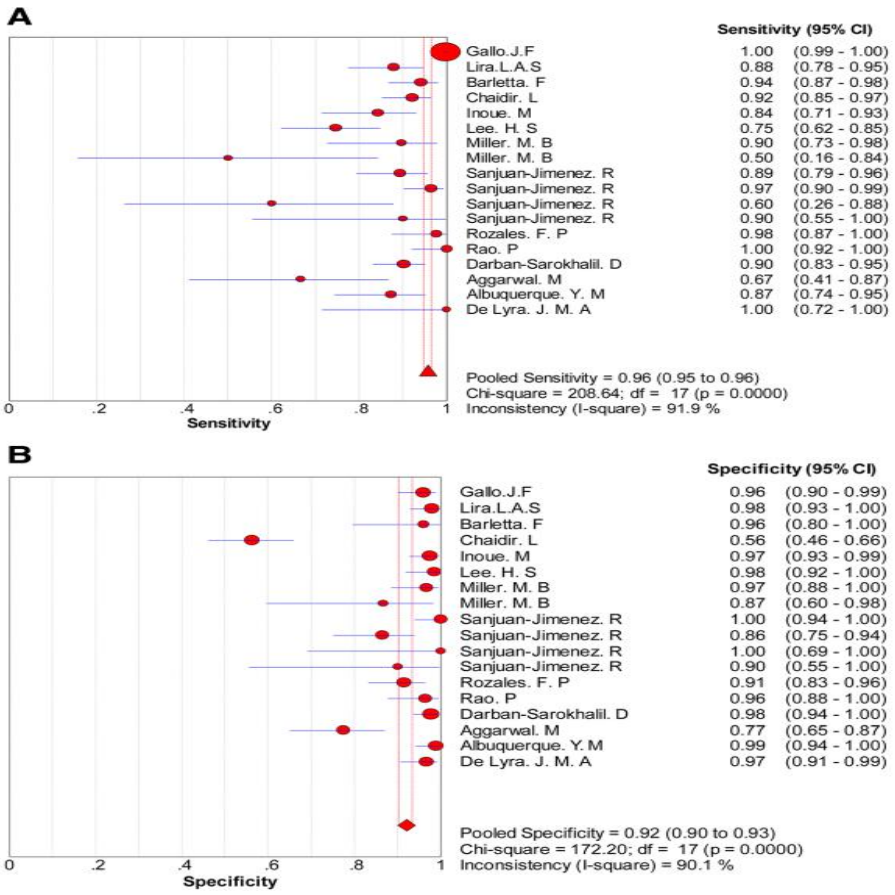

Fig. 3 Forest plot of specificity and sensitivity estimates. **a** Forest plot of sensitivity estimates and 95% confidence intervals (CI). The point estimates of sensitivity from each study are shown as solid circles. Error bars are 95% CI. Circles are proportional to study size. The pooled estimate is denoted by the diamond at the bottom. **b** Forest plot of specificity estimates and 95% CI. The point estimates of specificity from each study are shown as solid circles. Error bars are 95% CI. Circles are proportional to study size. The pooled estimate is denoted by the diamond at the bottom

Table 2 Pooled Summary Estimates of All Studies

Accuracy Measure	Pooled summary measure[a] (95% CI)	*P* value for heterogeneity[b]
Sensitivity	0.96 (0.95–0.96)	< 0.001
Specificity	0.92 (0.90–0.93)	< 0.001
Positive Likelihood Ratio (LR+)	16.90 (7.22–39.56)	< 0.001
Negative Likelihood Ratio (LR-)	0.11 (0.06–0.18)	< 0.001
Diagnostic Odds Ratio (DOR)	192.96 (68.46–543.90)	< 0.001

[a]Random effects model
[b]Chi-square or Fisher's exact test for heterogeneity

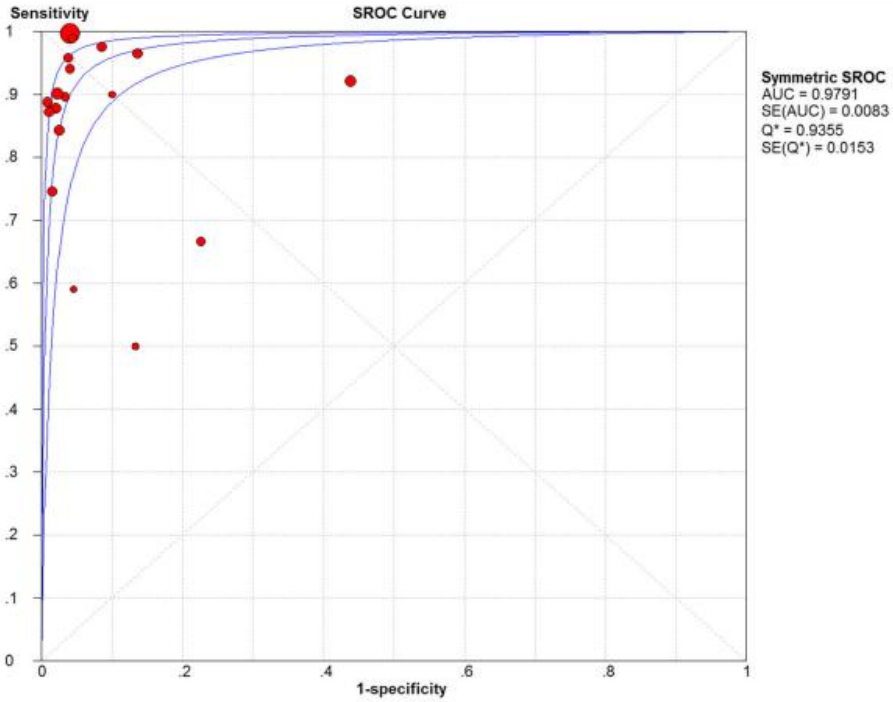

Fig. 4 Summary receiver operating characteristic curves for RT-PCR assays. Each solid circle represents each study in the meta-analysis. The curve is the regression line that summarizes the overall diagnostic accuracy. SROC = summary receiver operating characteristic; AUC = area under the curve; SE (AUC) = standard error of 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 Stratified analyses for the evaluation of heterogeneity in studies with real-time PCR assay

Subgroup (Number of studies)	Summary diagnostic odds ratio (95% CI)[a]	Chi^2 square test of heterogeneity	*P* value for heterogeneity[b]
Study design			
Cross-sectional (10)	403.18 (120.05–1354.05)	36.66	< 0.001
Case-control (8)	73.86 (20.40–267.48)	34.01	< 0.001
Target sequence			
IS6110 (11)	144.74 (51.24–408.86)	39.39	< 0.001
Other target (7)	297.17 (30.22–2921.73)	66.27	< 0.001
Specimen type			
Respiratory (11)	598.12 (269.12–1329.32)	19.09	0.039
Non-respiratory (5)	12.39 (6.67–22.73)	3.57	0.468
Both (2)	202.47 (64.68–633.83)	0.00	0.944
Region of study			
TB high-burden country (8)	281.86 (37.69–2107.75)	90.46	< 0.001
Other country (10)	160.73 (72.80–354.83)	15.17	0.086
Quality of study			
High-quality (7)	926.97 (303.59–2830.38)	12.83	0.046
Medium-quality (8)	76.77 (22.98–256.50)	26.65	< 0.001
Low-quality (3)	72.35 (4.47–1170.04)	19.07	< 0.001

[a]Random effects model

[b]chi-square or Fisher's exact test for heterogeneity; high-quality study: a study that had no domain with a high risk of bias and no domain with high applicability concerns; medium/moderate-quality study: a study that had domain with a unclear risk of bias or domain with unclear applicability concerns; low-quality study: a study that had a domain with a high risk of bias and domain with high applicability concerns

Table 4 Meta-regression analysis to determine sources of heterogeneity

Intercept	Coefficient	*P* value	Relative diagnostic odds ratio (RDOR)	95% confidence interval
Intercept	5.347	0.0000	–	–
Threshold (S)	0.169	0.5382	–	–
TB high-burden country vs. other country	0.756	0.3056	2.13	(0.46;9.96)
IS6110 vs. other target sequences	−0.812	0.2266	0.44	(0.11;1.77)
Cross-sectional design vs. case-control design	−0.759	0.5102	0.47	(0.04;5.45)
High-quality level vs moderate/low-quality level	1.175	0.0272	3.24	(1.17;9.00)
Respiratory specimens vs non-respiratory specimens /both	2.262	0.0025	9.60	(2.54;36.25)

High-quality study: a study that had no domain with a high risk of bias and no domain with high applicability concerns; medium/moderate-quality study: a study that had domain with an unclear risk of bias or domain with unclear applicability concerns; low-quality study: a study that had a domain with a high risk of bias and domain with high applicability concerns

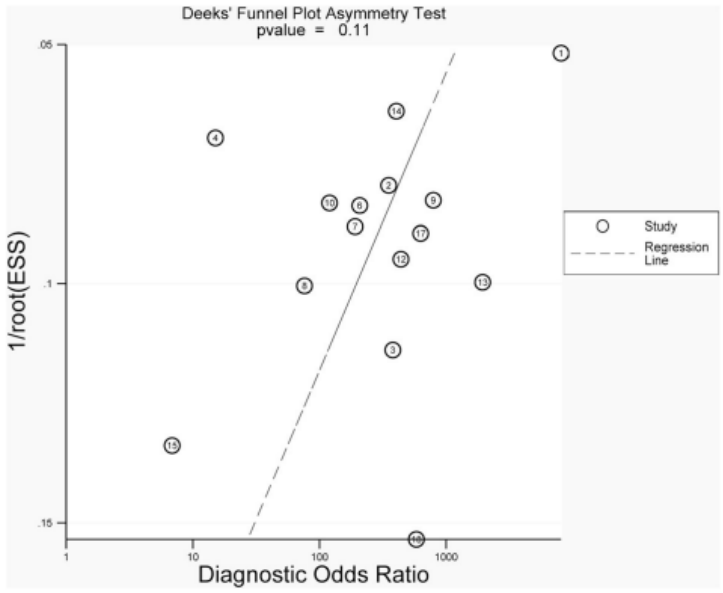

Fig. 5 A Deeks' funnel plot assessment test evaluated the potential publication bias for in-house RT-PCR assays. The plot shows the symmetric distribution of the log of diagnostic odds ratios against the inverse root of effective sample sizes (ESS), indicating the absence of any publication bias