A systematic review and meta-analysis of the diagnostic accuracy of nucleic acid amplification tests in cerebrospinal fluid for tuberculous meningitis

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Abstract

Introduction: Diagnosis of tuberculous meningitis (TBM) is difficult and poses a significant challenge to physicians worldwide. Recently, nucleic acid amplification (NAA) tests have shown promise for diagnosis of TBM, although performance has been variable. We undertook a systematic review and meta-analysis to evaluate the diagnostic accuracy of NAA tests in cerebrospinal fluid (CSF) samples against culture as the reference standard or a combined reference standard (CRS) for TBM.

Methods: We searched Embase, PubMed, Web of Science and the Cochrane library for the relevant records. QUADS-2 tool was used to assess the quality assessment of the studies. Diagnostic accuracy measures (i.e. sensitivity and specificity) were pooled with a random effects model. All Statistical analyses were performed with STATA version 14 (Stata Corporation, College Station, TX, USA), Meta-DiSc version 1.4 (Cochrane Colloquium, Barcelona, Spain) and RveMan version 5.3 (Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration).

Results: Sixty-three studies were included in final analysis, comprising 1381cases of confirmed TBM and 5712 non-TBM controls. These 63 studies were divided into two groups comprising 71 datasets (43 in-house tests and 28 commercial tests) that used culture as the reference standard and 24 datasets (21 in-house tests and 3 commercial tests) that used a CRS. Studies which used a culture reference standard had better pooled summary estimates compared to studies which used CRS. The overall pooled estimates of sensitivity, specificity, positive likelihood ratio (PLR) and negative likelihood ratio (NLR) of NAA tests against culture were 82% (95% CI: 75-87), 99% (95% CI: 98-99), 58.6 (35.3-97.3) and 0.19 (0.14-0.25), respectively. The pooled sensitivity, specificity, PLR and NLR of NAA tests against CRS were 68% (95% CI: 41-87), 98% (95% CI: 95-99), 36.5 (15.6-85.3) and 0.32 (0.15-0.70), respectively.

Conclusion: The analysis has demonstrated that the diagnostic accuracy of NAA tests is currently insufficient to replace culture as a lone diagnostic test. NAA tests may be used in combination with culture due to the advantage of time to result and in scenarios where culture tests are not feasible. Further work to improve NAA tests would benefit from standardized reference standards and the methodology.

Key words: Tuberculous Meningitis; Meta-analysis; diagnostic accuracy.

Introduction

Tuberculosis (TB) remains a global public-health problem with a high mortality rate. According to the World Health Organization (WHO), in 2017, TB caused an estimated 1.3 million deaths among human immunodeficiency virus (HIV)-negative people and an additional 300.000 deaths among HIV-positive people (1). Among all forms of TB, TB meningitis (TBM) is the most severe form, with substantial mortality (2-4). Approximately 30-40% of patients with TBM die despite anti-TB treatment (5, 6). Among HIV-infected patients the mortality rate of TBM may reach more than 60.0% (6). TBM caused by drug-resistant strains of *M. tuberculosis* has a mortality rate approaching 100% (7). The presenting clinical features of TBM are similar to those of other forms of sub-acute meningoencephalitides, making clinical diagnosis difficult and contributing to TBM's high mortality risk due to delay in starting treatment (8, 9). Consequently, delay in diagnosis and start of treatment have a negative impact on patients outcome (8). The cornerstones of TBM diagnosis remain the same as pulmonary TB: detection of acid-fast bacilli (AFB) by microscopy of the cerebrospinal fluid (CSF) and bacterial culture (9). Microscopy, although rapid and inexpensive, has very low sensitivity (approximately 10–20%) (8, 10). Mycobacterial culture is more sensitive (60–70%), but the results are not available for weeks (5, 11). In many cases, confirmation of TBM cannot be made on the basis of clinical and laboratory findings and empiric treatment is required (8). In the context of these limitations, several commercial and in-house nucleic acid amplification (NAA) techniques, have emerged and are in regular use to overcome the inadequacies of conventional methods of laboratory diagnosis (12). Beside their speed to diagnosis, ability to simultaneously detect drug resistance and reduce time to effective treatment, for areas without laboratory infrastructure for culture or high-quality microscopy, NAA, will have great advantages over the conventional methods. In the past decade, studies on the diagnostic accuracy of molecular methods for TBM have been published, but study design and the design of the NAA tests have varied, thus, the exact role of these tests remains uncertain (12-19). For example, the range of genetic targets used, capacity for on-demand or need for batch testing and time to final report are contributing factors for variation of NAA performance. Furthermore, newer tests (lipoarabinomannan lateral flow assay, adenosine deaminase) are currently being evaluated as alternatives to NAA test, hence the need for better data on the diagnostic accuracy of NAA tests to allow valid comparisons (20, 21). Furthermore, different case definitions and different reference standard test in studies make comparison of

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research findings difficult. A comprehensive meta-analysis of the diagnostic accuracy of NAA tests for TBM was published in 2003, which used microbiological diagnosis, microbiological plus clinical diagnosis and clinical diagnosis as three different reference standards. Newly developed commercially available tests such as GeneXpert MTB/RIF were not available at that time (12). In 2014, a WHO systematic review of GeneXpert found a pooled sensitivity of 80.5% (95% CI 59.0–92.2%) against culture and 62.8% (95% CI 47.7–75.8%) against combined reference standard (CRS) for extrapulmonary TB (22). These findings led to a WHO recommendation for use of GeneXpert as a first line test for detection of extrapulmonary TB and widespread uptake of use worldwide (10, 23). Yet, other NAA tests have not been systemically investigated and their performance compared to GeneXpert and the reengineered Xpert Ultra is not clear. Additionally, subsequent, substantial studies of both GeneXpert, and the Xpert Ultra have been published since the WHO systematic review. Therefore, this systematic review was performed to evaluate the diagnostic accuracy of NAA tests for TBM based on two reference standard testes; culture confirmed TBM and CRS.

Methods

Search strategy

We searched all studies published up to November 11, 2018 from the following databases: Embase, PubMed, Web of Science and the Cochrane library. Search terms used were: “Mycobacterium tuberculosis”, “tuberculosis”, “tuberculous meningitis”, “meningitis”, “cerebrospinal fluid”, “CSF”, “molecular diagnostic techniques”, “nucleic acid amplification”, “diagnosis”, “Polymerase Chain Reaction”, “PCR”, “loop mediated isothermal amplification”, “LAMP”, “GeneXpert”, “Xpert”, “ligase chain reaction”, “LCx”, “Amplicor”, “ProbeTec”, “Gen-probe”, “GenoType MTBDR”, “Cobas”, “Roche”, “Abbott” and “Cepheid”. In addition, we searched references of included articles to find relevant studies. Only studies written in English were selected. This study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (24).

Study selection

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The studies found through databases that were duplicates were removed using EndNote X7 (Thomson Reuters, New York, NY, USA). Records were initially screened by title and abstract by two independent reviewers (AP, MJN) to exclude those not related to the current study. The full-text of potentially eligible records was retrieved and examined. Any discrepancies were resolved by consensus.

Inclusion criteria

Studies were included if they report a comparison of an NAA test against a reference standard and provide data necessary for the computation of both sensitivity and specificity. We used the TBM definition by Thwaites diagnostic index and Marais criteria (8, 25). Briefly, Confirmed TBM was defined as any patient with positive culture for TBM. Likewise, CRS was definite as any patients who fulfill clinical criteria plus one or more of the following: acid-fast bacilli seen in the CSF; Mycobacterium tuberculosis cultured from CSF; or CSF-positive NAA test. Two reviewers (AP and MJN) independently judged study eligibility. Disagreements were resolved by consensus.

Exclusion criteria

Studies were excluded if they: did not report confirmed and/or suspected TBM based on Thwaites and Marais diagnostic criteria, did not report sufficient data for computation of sensitivity and specificity and did not contain enough samples (≤10 CSF samples).

Data extraction

The following items were extracted from each article: first author, year of publication, study time, study location, type of NAA test used, reference standard used, number of confirmed TBM cases, number of suspected TBM cases and number of non-TBM (controls). Two reviewers (AP and MJN) independently extracted data and differences were resolved by consensus.

Quality assessment

The methodological quality of the studies was assessed using the QUADAS-2 checklist (26).

Analysis

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Statistical analyses were performed with STATA (version 14 IC; Stata Corporation, College Station, TX, USA), Meta-DiSc 1.4 for Windows (Cochrane Colloquium, Barcelona, Spain) and RveMan Version 5.3 (Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration). The pooled sensitivity, specificity, and diagnostic odds ratio (DOR) with 95% confidence intervals between NAA tests and reference standard were assessed. A random effects model was used to pool the estimated effects. Diagnostic accuracy measures [i.e. the summary receiver operating characteristic (SROC) curve, the summary positive likelihood ratios (PLR), negative likelihood ratios (NLR) and DOR] were calculated. A value of pooled PLR greater than 10 and of pooled NLR less than 0.1 were noted as providing convincing diagnostic evidence (27, 28). The heterogeneity among the studies was assessed using Chi-square test and I-square statistics. To identify the risk of publication bias, Deek's test was used, based on parametric linear regression methods (29). Subgroup analysis was conducted using several study characteristics separately.

Results

Figure 1 summarizes the study selection process. Briefly, we retrieved data from 63 selected articles comprising 1381 confirmed TBM cases and 5712 non-TBM controls. These 63 studies were divided into two groups comprising 71 datasets (43 in-house tests and 28 commercial tests) that used culture as the reference standard and 24 datasets (21 in-house tests and 3 commercial tests) that used a CRS. Characteristics of the included studies are described in Table 1. The studies were conducted in 22 different countries: India was the most frequently represented country (28 out of 63, 44.4%).

Risk of bias assessment

Based on the QUDAS-2 tool, all included records were identified as having a low risk of bias, thereby increasing the strength of scientific evidence of the current study (Figure 2). The quality assessment for each included study is provided in Figure S1.

Overall diagnostic accuracy of NAA tests against culture

The overall pooled estimates of sensitivity, specificity, PLR, NLR and DOR of NAA tests against culture were 82% (95% CI: 75-87), 99% (95% CI: 98-99), 58.6 (35.3-97.3), 0.19 (0.14-0.25) and 314 (169-584), respectively (Table 2, Figure 3). The SROC plot showed an AUC of 98% (96-99) (Figure 4). The Deek's test result indicated low likelihood for publication bias (P= 0.01).

Diagnostic accuracy of in-house tests against culture

The pooled sensitivity and specificity estimates of in-house NAA tests against culture were 87% (80-92) and 99% (97-99). The PLR, NLR, DOR and AUC estimates were found to be 64.6 (28.4-147.0), 0.13 (0.08-0.20), 372 (165-839) and 98% (97-99), respectively (Table 2, Figure S2, S3).

Diagnostic accuracy of commercial tests against culture

The pooled sensitivity and specificity estimates of commercial tests against culture were 67% (58-75) and 99% (98-99), respectively. The PLR, NLR, DOR and AUC estimates were found to be 46.1 (28.3-75.0), 0.33 (0.25-0.43), 139 (71-274) and 98% (96-99), respectively (Table 2, Figure S4, S5).

Overall diagnostic accuracy of NAA tests against CRS

The overall pooled estimates of sensitivity, specificity, PLR, NLR, DOR and AUC of NAA tests against CRS were 68% (95% CI: 41-87), 98% (95% CI: 95-99), 36.5 (15.6-85.3), 0.32 (0.15-0.70), 113 (39-331) and 98% (96-99) respectively (Table 2, Figure 5, 6). There was no evidence of publication bias (Deek's Test P value was 0.01).

Diagnostic accuracy of in-house tests against CRS

The pooled sensitivity of in-house NAA tests against CRS was 68% (38-88), and the pooled specificity was 98% (95-1.00) (Table 2, Figure S6, S7). The PLR, NLR, DOR and AUC estimates were 44.4 (16.0-123.2), 0.32 (0.14-0.75), 138 (41-468) and 98% (96-99), respectively.

Diagnostic accuracy of commercial tests against CRS

The pooled sensitivity of commercial NAA tests against CRS was 53% (33.4-73.4), and the pooled specificity was 90% (82-95). The PLR, NLR, DOR and AUC estimates were 70 (40.0-124.2), 0.57 (0.24-0.31), 21 (4.2-104.0) and 94% (90-97), respectively (Table 2).

Between-group comparisons

In group with culture reference standard, NAA tests revealed better pooled summary estimates [sensitivity=82% (75-87), specificity=99% (95% CI: 98-99), 58.6 (35.3-97.3), NLR = 0.19 (0.14-

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0.25), DOR=314 (169-584), AUC=98% (96-99)] as compared to CRS group [sensitivity=68% (95% CI: 41-87), specificity=98% (95% CI: 95-99), PLR=36.5 (15.6-85.3), NLR=0.32 (0.15-0.70), DOR=113 (39-331), AUC=98% (96-99) (Table 2).

In group with culture reference standard, in-house test has higher sensitivity, PLR and DOR, comparable specificity and AUC but lower NLR as compared to commercial test. Likewise, in CRS group, in-house test has higher sensitivity, specificity and DOR, but lower PLR, NLR as compared to commercial test.

Subgroup analysis

Table 3 shows the subgroup analysis of the studies based on different NAA tests.

Discussion

Early and accurate diagnosis of TBM is crucial to reduce morbidity and mortality. However, different case definitions and different reference standards used in various studies makes comparison of research findings difficult and limits the management of disease. In the present study, the sensitivity and specificity of different NAA tests was assessed based on two most reliable reference standard tests (culture confirmed TBM and CRS). Based on the results obtained from our analysis we identified that the studies with culture reference standard had better summary estimates as compared to studies used CRS as reference standard. Thus, the inclusion of confirmed TBM as the main reference standard test could be applied in diagnosing algorithms which would lead to better management of TBM.

Based on our analysis, the pooled estimates of sensitivity, specificity, PLR, NLR, DOR and AUC of in-house NAA tests against culture were 87% (80-92), 99% (97-99), 64.6 (28.4-147.0), 0.13 (0.08-0.20), 372 (165-839) and 98% (96-99), respectively. Likewise, the pooled sensitivity, specificity, PLR, NLR, DOR and AUC for commercial NAA tests against culture were 67% (58-75), 99% (98-99), 46.1 (28.3-75.0), 0.33 (0.25-0.43), 139 (71-274) and 98% (96-99), respectively.

Although the sensitivity of in-house tests was higher than the commercial NAA tests, the decontamination process, the DNA extraction protocol, target genes adopted, presence of PCR inhibitors and the quality of reaction materials are among the factors that may lead to bias in the

in-house tests. Thus, while these results are encouraging, in-house tests are unlikely to be a widespread answer for accurate diagnosis of TBM.

The PLR of commercial tests was 46.1, suggesting that patients with TBM have a 46-fold higher chance of being NAA test-positive compared with patients without TBM. In contrast to findings from a prior systematic review performed in 2003, we found higher sensitivity of the commercial tests (12). Furthermore, when comparing our summary estimates of commercial tests to the previous meta-analysis, the NLR is lower in our study, (0.33 versus 0.44), but not low enough to rule out TBM with great confidence (12). Thus, our results suggest that a negative commercial NAA test should not be used alone as a justification to rule out TBM (30). To rule out TBM, the results of NAA tests should be confirmed by conventional tests such as culture and smear (12). By contrast, our meta-analysis indicated that a positive commercial NAA result provides a definite TBM diagnosis (12). Despite suboptimal sensitivity, the rapid turnaround time of commercial NAA tests compared to culture enhances its role in the early accurate diagnosis of TBM. In the management of TBM, this rapidity is of great relevance and may improve outcomes (12).

Recently, GeneXpert MTB/RIF has been a major breakthrough in the diagnosis of TB Meningitis (10, 13, 31). Likewise, based on the results of a systematic review published in 2014, Xpert was recommended as the preferred test for diagnosis of TB meningitis by the WHO (22, 32). In our analysis, the sensitivity and specificity of GeneXpert MTB/RIF assay was 67% and 98%, respectively, against culture. By comparison, the 2014 meta-analysis by Denkinger and colleagues reported a pooled sensitivity of 80.5% against culture (22). Cost-effectiveness analysis of the use of the GeneXpert MTB/RIF assay has been completed and suggests that this technology is likely to be a highly cost-effective method of TB diagnosis; however, these analyses were not TBM specific (33-36).

More recently, Bahr et al evaluated the diagnostic performance of the new GeneXpert MTB/RIF Ultra (Xpert Ultra) for TBM (23). They found Xpert Ultra had 95% sensitivity for TBM compared to a CRS of any microbiologic test being positive. When Xpert Ultra was excluded from the reference standard, sensitivity was 70%. In both analyses, Xpert Ultra's sensitivity was higher than either Xpert or culture, leading the WHO to recommend Xpert Ultra as the initial test for TBM (23, 32, 37).

Some limitations of this study should be taken into consideration. First, heterogeneity exists among the included studies. To explore the heterogeneity of studies, we conducted subgroup, meta-regression and sensitivity analyses. The subgroup and meta-regression analyses found that variables such as NAA techniques and standard tests could be probable reasons of heterogeneity. Second, we could not address the effect of factors such as sample volume, processing steps, amplification protocols, expertise with NAA tests and laboratory infrastructure on the accuracy of NAA tests due to a high level of variability in these factors and/or reporting of these factors in the studies. Finally, as with any systematic review, limitations associated with potential publication bias should be considered.

Conclusions

The analysis has demonstrated that the diagnostic accuracy of NAA tests is currently insufficient to replace culture for diagnosis of TBM as a singular test. However, NAA test use in combination with culture due to more timely results from NAA tests and their ability to detect dead bacilli should be considered when feasible.

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Authors' contributions:

Study design: AP, NCB, TDM and MJN.
Literature search, data collection, data analysis and data interpretation: AP, MJN, SMR
Writing: AP, MJN
Manuscript editing: AP, MJN, NCB, TDM.

Conflict of interest:

We declare that we have no conflicts of interest

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Ethics committee approval:

Not applicable.

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Figures:

Figure 1. Flow diagram of literature search and study selection.

Figure 2. QUADAS-2 assessments of included studies.
Patient Selection: Describe methods of patient selection; Index Text: Describe the index test and how it was conducted and interpreted; Reference Standard: Describe the reference standard (gold standard test) and how it was conducted and interpreted; Flow and Timing: Describe any patients who did not receive the index tests or reference standard or who were excluded from the 2 × 2 table, and describe the interval and any interventions between index tests and the reference standard (26).

Figure 3. Paired forest plots of pooled sensitivity and specificity of NAA tests against culture.

Figure 4. Summary receiver operating characteristic (SROC) plot for NAA tests against culture.
The SROC plot shows summary of test performance, visual assessment of threshold effect, and heterogeneity of data in ROC space between sensitivity and specificity; each circle in the SROC plot represents a single study, summary operating sensitivity specificity, and SROC curve with both confidence and prediction regions. The dashed line that is around the pooled point estimate shows 95% confidence region. The area under the curve (AUC), acts as an overall measure for test performance. Particularly, when AUC would be between, 0.8 to 1, the accuracy is relatively high. As a matter of fact, AUC was 0.52 in this report which represented a relatively moderate level of accuracy. If SROC curve was in the upper left corner it would showed the best combination of sensitivity and specificity for the diagnostic test.

Figure 5. Paired forest plots of pooled sensitivity and specificity of NAA tests against CRS.

Figure 6. Summary receiver operating characteristic (SROC) plot for NAA tests against CRS.

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615 Table 1. Characterization of included studies.

First author	Country	Published year	NAA test	Diagnostic method	Gene target	Reference standard	No. of confirmed TBM	No. of Non-TBM (Control)	Study design	Consecutive sampling	Data collection	Blinded
Afroze*(38)	India	2008	In-house	Conventional PCR	MPB64	CRS	27	10	CC	NM	R	Yes
Baveja (39)	India	2009	In-house	Conventional PCR	IS6110	CRS	22	78	CS	Yes	P	NM
Berwal (40)	India	2017	In-house	Conventional PCR	IS6110	CRS	26	48	CS	NM	P	NM
Bhigjee1 (41)	South Africa	2007	In-house	Conventional PCR	IS6110	Culture	20	24	CS	NM	P	Yes
Bhigjee2 (41)	South Africa	2007	In-house	Conventional PCR	MPB64	Culture	20	24	CS	NM	P	Yes
Bhigjee3 (41)	South Africa	2007	In-house	Conventional PCR	Pt8/Pt9	Culture	20	24	CS	NM	P	Yes
Bhigjee4 (41)	South Africa	2007	In-house	Real-time PCR	IS6110	Culture	20	24	CS	NM	P	Yes
Brienze1 (42)	Brazil	2001	In-house	Nested PCR	MPB64	CRS	15	50	CS	NM	P	NM
Caws (43)	United Kingdom	2000	In-house	Conventional PCR	IS6110	Culture	4	105	CS	Yes	P	NM
Chaidir (44)	Indonesia	2012	In-house	Real-time PCR	IS6110	Culture	102	105	CS	Yes	P	Yes
Desai1 (45)	India	2006	In-house	Conventional PCR (QIAmp protocol)	IS6110	CRS	8	27	CS	Yes	P	NM
Desai2 (45)	India	2006	In-house	Conventional PCR (CTAB protocol)	IS6110	CRS	8	27	CS	Yes	P	NM
Deshpande (15)	India	2007	In-house	Conventional PCR	IS6110	CRS	35	29	CC	NM	P	NM
Haldar1 (46)	India	2009	In-house	Conventional PCR (filtrate protocol)	IS6110	Culture	10	86	CS	NM	NM	Yes
Haldar2 (46)	India	2009	In-house	Conventional PCR (sediment protocol)	IS6110	Culture	10	86	CS	NM	NM	Yes
Haldar3 (46)	India	2009	In-house	Conventional PCR (filtrate protocol)	devR	Culture	10	86	CS	NM	NM	Yes
Haldar4 (46)	India	2009	In-house	Conventional PCR (sediment protocol)	devR	Culture	10	86	CS	NM	NM	Yes
Haldar5 (46)	India	2009	In-house	Real-time PCR (filtrate protocol)	devR	Culture	10	86	CS	NM	NM	Yes
Haldar6 (46)	India	2009	In-house	Real-time PCR (sediment protocol)	devR	Culture	10	86	CS	NM	NM	Yes
Haldar7 (15)	India	2012	In-house	Conventional PCR	devR	Culture	29	338	CS	NM	P	Yes
Juan (47)	Spain	2006	In-house	Conventional PCR	IS6110	CRS	12	59	CS	Yes	P	NM
Kulkarni1*(18)	India	2005	In-house	Conventional PCR (ETBR protpcol)	Protein b	CRS	30	30	CS	NM	NM	Yes
Kulkarni2 (18)	India	2005	In-house	Conventional PCR (southern protocol)	Protein b	CRS	30	30	CS	NM	NM	Yes
Lekhak1*(48)	Nepal	2016	In-house	Conventional PCR	IS6110	CRS	37	75	CS	NM	NM	NM

Lekhak2 (48)	Nepal	2016	In-house	Conventional PCR	MPB64	CRS	37	75	CS	NM	NM	NM
Michael (49)	India	2002	In-house	Conventional PCR	IS6110	Culture	17	68	CS	NM	R	Yes
Miorner (50)	India	1995	In-house	Conventional PCR	IS6110	Culture	6	34	CC	NM	NM	NM
Modi1 (51)	India	2016	In-house	Conventional PCR	IS6110	Culture	50	100	CS	NM	NM	NM
Modi2 (51)	India	2016	In-house	LAMP PCR	IS6110	Culture	50	100	CS	NM	NM	NM
Modi3 (51)	India	2016	In-house	LAMP PCR	MPB64	Culture	50	100	CS	NM	NM	NM
Nagdev1 (52)	India	2010	In-house	Nested PCR	IS6110	Culture	1	13	CC	NM	NM	NM
Nagdev2 (53)	India	2010'	In-house	Conventional PCR	IS6110	Culture	13	139	CC	NM	P	NM
Nagdev3*(54)	India	2011	In-house	Nested PCR	IS6110	CRS	17	10	CC	NM	R	NM
Nagdev4 (54)	India	2011	In-house	LAMP PCR	IS6110	CRS	17	10	CC	NM	R	NM
Nagdev5 (55)	India	2015	In-house	Multiplex PCR	16s rDNA	Culture	8	85	CS	NM	P	NM
Nagdev6 (55)	India	2015	In-house	Multiplex PCR	IS6110	Culture	8	85	CS	NM	P	NM
Narayanan1 (56)	India	2001	In-house	Conventional PCR	IS6110	Culture	20	8	CS	NM	NM	NM
Narayanan2 (56)	India	2001	In-house	Conventional PCR	TRC4	Culture	20	8	CS	NM	NM	NM
Nguyen (57)	Vietnam	1996	In-house	Conventional PCR	IS6110	Culture	17	32	CS	Yes	R	Yes
Palomo1*(58)	Brazil	2017	In-house	Conventional PCR	IS6110	CRS	35	65	CS	NM	NM	NM
Palomo2 (58)	Brazil	2017	In-house	Conventional PCR	MBP64	CRS	35	65	CS	NM	NM	NM
Palomo3 (58)	Brazil	2017	In-house	Conventional PCR	hsp65	CRS	35	65	CS	NM	NM	NM
Portillo (59)	Mexico	2000	In-house	Conventional PCR	IS6110	Culture	13	113	CS	NM	NM	NM
Quan (16)	China	2006	In-house	Conventional PCR	IS6110	Culture	3	49	CC	NM	NM	NM
Rafi1 (14)	India	2007	In-house	Conventional PCR	IS6110	Culture	45	75	CS	NM	R	Yes
Rafi2 (14)	India	2007	In-house	Nested PCR	MPB64	Culture	45	75	CS	NM	R	Yes
Rafi3 (14)	India	2007	In-house	Nested PCR	65 Kda	Culture	45	75	CS	NM	R	Yes
Rafi4 (60)	India	2007	In-house	Conventional PCR	IS6110	Culture	136	268	CS	NM	P	Yes
Rana (61)	India	2010	In-house	Conventional PCR	IS6110	Culture	5	37	CS	NM	P	NM
Rios-Sarabia1*(62)	Mexico	2016	In-house	Multiplex PCR	Protein b	CRS	50	50	CC	Yes	P	Yes
Rios-Sarabia2 (62)	Mexico	2016	In-house	Multiplex PCR	IS6110	CRS	50	50	CC	Yes	P	Yes
Rios-Sarabia3 (62)	Mexico	2016	In-house	Multiplex PCR	MPB40	CRS	50	50	CC	Yes	P	Yes
Rios-Sarabia4 (62)	Mexico	2016	In-house	Nested PCR	MPB40	CRS	50	50	CC	Yes	P	Yes
Sastry (63)	India	2013	In-house	Nested PCR	IS6110	Culture	2	33	CC	Yes	P	NM
Shankar (64)	India	1991	In-house	Conventional PCR	MPB64	Culture	4	51	CS	NM	NM	NM
Sharma1 (65)	India	2010	In-house	Conventional PCR	Protein b	Culture	10	40	CS	NM	NM	NM
Sharma2 (66)	India	2011	In-house	Multiplex PCR	IS6110	Culture	18	100	CS	Yes	NM	Yes

Sharma3 (66)	India	2011	In-house	Multiplex PCR	MPB64	Culture	18	100	CS	Yes	NM	Yes
Sharma4 (66)	India	2011	In-house	Multiplex PCR	Protein b	Culture	18	100	CS	Yes	NM	Yes
Sharma5 (67)	India	2012	In-house	Conventional PCR	MPB64	Culture	9	40	CS	NM	P	NM
Sharma6 (68)	India	2015	In-house	Real-time PCR	IS6110	Culture	12	120	CS	NM	NM	NM
Sharma7 (68)	India	2015	In-house	Real-time PCR	MPB64	Culture	12	120	CS	NM	NM	NM
Sharma8 (68)	India	2015	In-house	Real-time PCR	rpoB	Culture	12	120	CS	NM	NM	NM
Sumi (69)	India	2002	In-house	Conventional PCR	IS6110	Culture	8	45	CC	NM	NM	Yes
Bahr1 (10)	Uganda	2015	Commercial	GeneXpert	rpoB	Culture	18	89	CS	NM	NM	NM
Bahr2 (23)	Uganda	2018	Commercial	GeneXpert Ultra	rpoB, IS6110, IS1081	Culture	22	107	CS	NM	P	NM
Baker (70)	United States	2002	Commercial	Gen-probe MTD	16s RNA	Culture	5	24	CS	NM	NM	Yes
Bonington (17)	South Africa	2000	Commercial	Cobas Amplicor MTB	16s RNA	Culture	8	29	CS	NM	P	NM
Brienze2 (42)	Brazil	2001	Commercial	Cobas Amplicor MTB	16s RNA	CRS	11	17	CS	NM	P	NM
Causse1 (71)	Spain	2011	Commercial	GeneXpert	rpoB	Culture	6	299	CS	Yes	NM	NM
Causse2 (71)	Spain	2011	Commercial	Cobas Amplicor MTB	16s RNA	Culture	6	299	CS	Yes	NM	NM
Chedore (72)	Canada	2002	Commercial	Gen-probe MTD	16s RNA	Culture	16	295	CS	NM	NM	NM
Chua (73)	Singapore	2005	Commercial	Abbott LCx ligase chain reaction	Protein b	Culture	6	36	CC	NM	P	NM
Cox (20)	Uganda	2015	Commercial	GeneXpert	rpoB	CRS	8	69	CS	NM	NM	NM
Johansen (74)	Denmark	2004	Commercial	ProbeTec	IS6110	Culture	13	88	CS	NM	NM	NM
Jönsson (75)	Sweden	2003	Commercial	Cobas Amplicor MTB	16s RNA	Culture	9	145	CS	Yes	R	NM
Khan (76)	Pakistan	2018	Commercial	GeneXpert	rpoB	Culture	12	47	CS	NM	NM	NM
Lang (1)	Dominican Republic	1998	Commercial	Gen-probe MTD	16s RNA	Culture	5	60	CS	Yes	P	NM
Li (77)	China	2017	Commercial	GeneXpert	rpoB	Culture	4	70	CS	Yes	NM	NM
Malbruny (78)	France	2011	Commercial	GeneXpert	rpoB	Culture	1	14	CS	Yes	P	NM
Moure (79)	Spain	2011	Commercial	GeneXpert	rpoB	Culture	2	12	CS	NM	NM	NM
Nhu (13)	Vietnam	2013	Commercial	GeneXpert	rpoB	Culture	151	197	CS	Yes	P	Yes
Patel1 (80)	South Africa	2014	Commercial	GeneXpert	rpoB	Culture	31	53	CS	Yes	P	Yes
Patel2 (80)	South Africa	2014	Commercial	Cobas Amplicor MTB	16s RNA	Culture	31	53	CS	Yes	P	Yes
Pink (81)	United Kingdom	2016	Commercial	GeneXpert	rpoB	Culture	37	703	CS	NM	NM	NM
Rakotoarivelo (82)	Madagascar	2018	Commercial	GeneXpert	rpoB	Culture	13	31	CS	NM	NM	NM

Rufai (83)	India	2017	Commercial	GeneXpert	rpoB	Culture	49	212	CS	NM	NM	NM
Solomons1 (84)	South Africa	2015	Commercial	GenoType MTBDRplus	INH, RIF	Culture	13	46	CS	Yes	P	NM
Solomons2 (84)	South Africa	2015	Commercial	GeneXpert	rpoB	Culture	13	46	CS	Yes	P	NM
Thwaites (11)	Vietnam	2004	Commercial	Gen-probe MTD	16s RNA	Culture	42	79	CS	Yes	P	Yes
Tortoli (85)	Italy	2012	Commercial	GeneXpert	rpoB	Culture	13	120	CS	NM	R	Yes
Vadwai1 (86)	India	2011	Commercial	GeneXpert	rpoB	CRS	7	15	CS	NM	NM	Yes
Vadwai2 (86)	India	2011	Commercial	GeneXpert	rpoB	Culture	3	19	CS	NM	NM	Yes
Wang (87)	China	2016	Commercial	GeneXpert	rpoB	Culture	13	188	CS	NM	P	Yes
Zmak (88)	Croatia	2013	Commercial	GeneXpert	rpoB	Culture	1	45	CS	NM	NM	NM

*These studies did not used culture to define confirm TBM.

CRS: combined reference standard, P: prospective, R: retrospective, CS: cross-sectional, CC: case-control, NM: Not mentioned

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641 Table 2. Summary measures of test accuracy for all studies, commercial, and in-house tests.

Test property		Sensitivity (95% CI, I^2)	Specificity (95% CI, I^2)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC (95% CI)
All studies (63 studies)	Culture (71 datasets with 1492 TBM cases)	82% (75-87, 82.4%)	99% (98-99, 85.0%)	58.6 (35.3-97.3)	0.19 (0.14-0.25)	314 (169-584)	98% (96-99)
	CRS (24 datasets with 652 TBM cases)	68% (41-87, 83.6%)	98% (95-99, 76.2%)	36.5 (15.6-85.3)	0.32 (0.15-0.70)	113 (39-331)	98% (96-99)
Culture (71 datasets)	In-house tests (43 datasets with 950 TBM cases)	87% (80-92, 82.0%)	99% (97-99, 88.5%)	64.6 (28.4-147.0)	0.13 (0.08-0.20)	372 (165-839)	98% (97-99)
	Commercial tests (28 datasets with 543 TBM cases)	67% (58-75, 64.8%)	99% (98-99, 48.3%)	46.1 (28.3-75.0)	0.33 (0.25-0.43)	139 (71-274)	98% (96-99)
CRS (24 datasets)	In-house tests (21 datasets with 626 TBM cases)	68% (38-88, 83.5%)	98% (95-100, 78.0%)	44.4 (16.0-123.2)	0.32 (0.14-0.75)	138 (41-468)	98% (96-99)
	Commercial tests (3 datasets with 26 TBM cases)	53% (33-73, 84.7%)	90% (82-95, 52.2%)	70.0 (40.0-124.2)	0.57 (0.24-0.31)	21 (4.2-104)	94% (90-97)

642 CRS: combined reference standard, PLR: positive likelihood ratio, NLR: negative likelihood ratio, DOR: diagnostic odds ratio, AUC: area under the curve.

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651 Table 3. Subgroup analysis of studies based on different NAA tests.

Reference standard	Subgroup	Subgroup by method	No. of datasets	Sensitivity (95% CI)	Specificity (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC (95% CI)
Culture	In-house	Conventional PCR (IS6110 gene)	18	87% (77-93)	98% (94-99)	39.5 (15.7-77.1)	0.13 (0.07-0.25)	307 (106- 888)	98 (96-99)
		Conventional PCR (MPB64 gene)	4	92% (81- 97)	98% (78-99)	52.0 (3.4-778.4)	0.08 (0.03-0.20)	275 (42-1814)	93 (91-95)
		Nested PCR	4	82% (46- 96)	92% (88-95)	10.7 (5.9-19.4)	0.19 (0.05- 0.79)	55 (9-339)	93 (91-95)
		Real-time PCR	7	84% (71-92)	100% (45-100)	44.0 (5.7- 335.4)	0.16 (0.08,0.65)	255 (40-607)	93 (91-95)
		LAMP PCR	2	93% (88 -97)	100% (98 -100)	68.8 (0.68-925.8)	0.07 (0.03-0.13)	-	-
	Commercial	Cobas Amplicor MTB	4	48% (35- 61)	98% (97-99)	25.3 (12.9-49.7)	0.53 (0.41-0.68)	48 (21-109)	94 (91-95)
		GeneXpert	16	61% (52-70)	99% (97-99)	42.0 (20.6-85.2)	0.39 (0.31- 0.50)	107 (64-251)	92 (89-94)
		Gen-probe MTD	4	86% (52-97)	99% (95-100)	92.4 (14.8-577.6)	0.15 (0.03- 0.63)	634 (31-1299)	99 (98-100)
CRS	In-house	Conventional PCR (IS6110 gene)	9	87% (46- 98)	98% (88-100)	39.2 (7.8-197.8)	0.13 (0.02- 0.78)	119 (42-332)	99 (97-99)
		Conventional PCR (MPB64 gene)	4	27% (02-85)	99% (91-100)	35.9 (1.7-751.1)	0.74 (0.36-1.52)	45 (8-249)	99 (97-99)
		Nested PCR	3	80% (70 - 88)	95% (0.89-98)	11.9 (5.3-6.7)	0.23 (0.05-1.02)	86 (7-1049)	97 (93-99)
	Commercial	GeneXpert	2	66% (38- 88)	89% (80-95)	7.0 (3.8-12.8)	0.23 (0.00- 19.53)	-	-

652 CRS: combined reference standard, PLR: positive likelihood ratio, NLR: negative likelihood ratio, DOR: diagnostic odds ratio, AUC: area under the curve.

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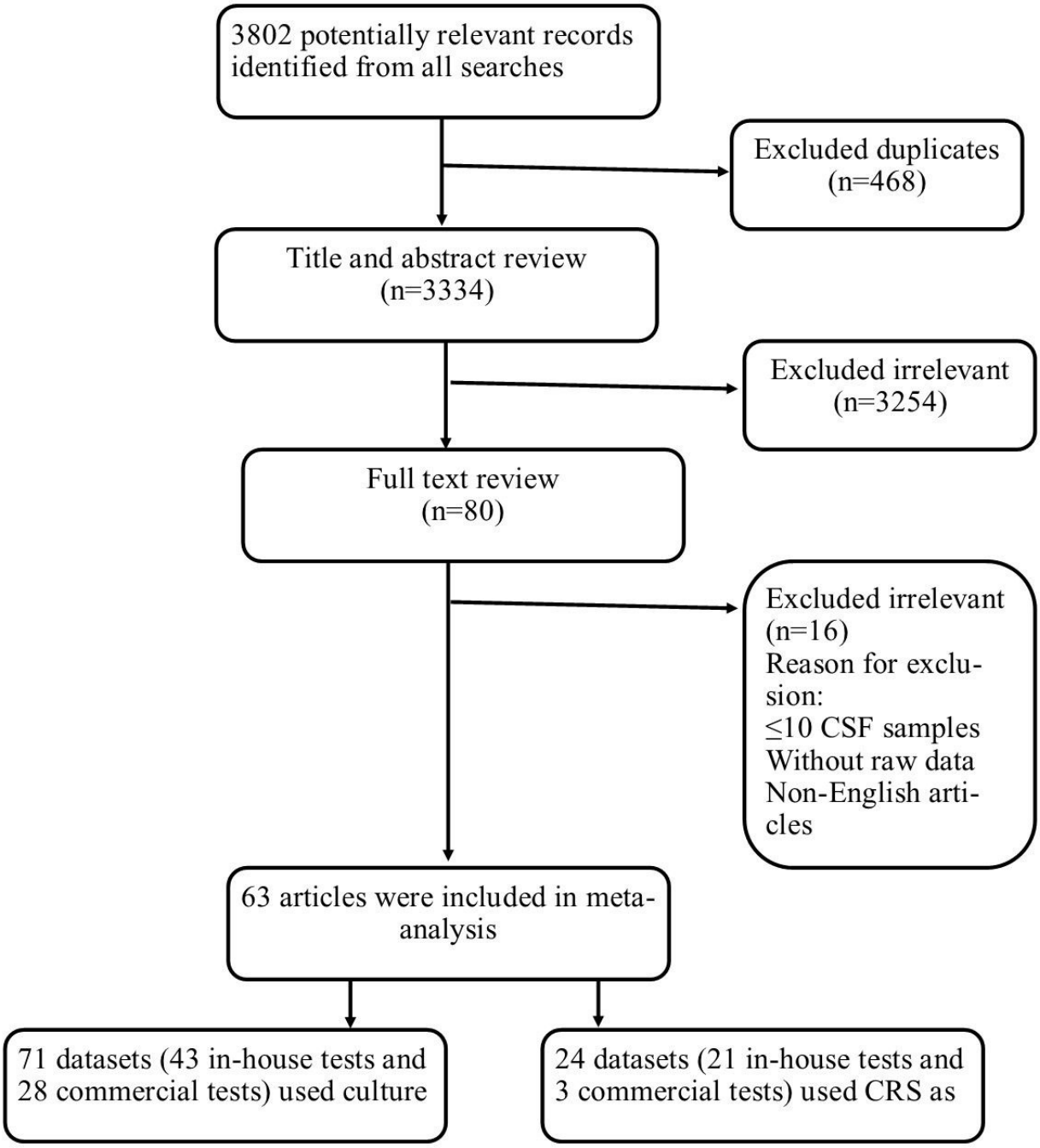

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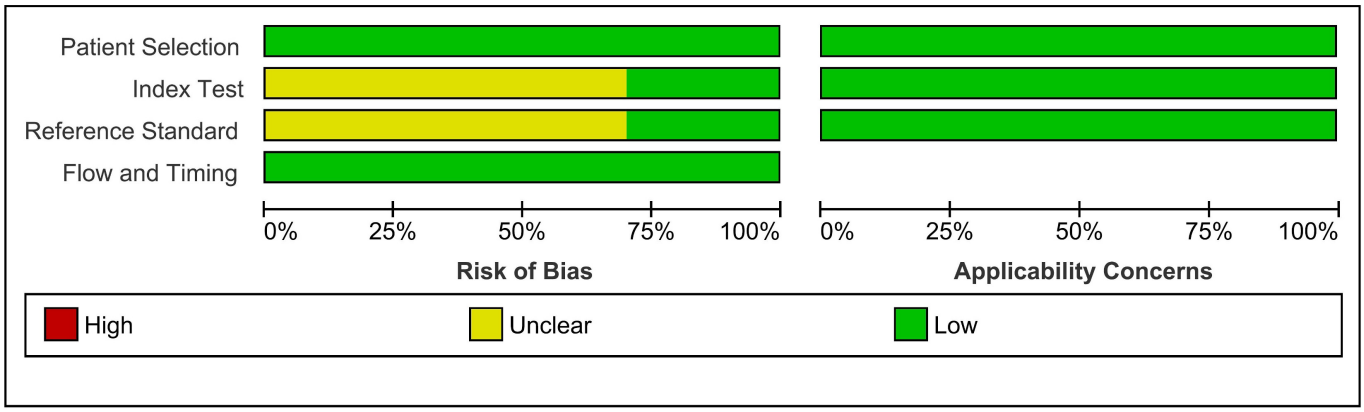

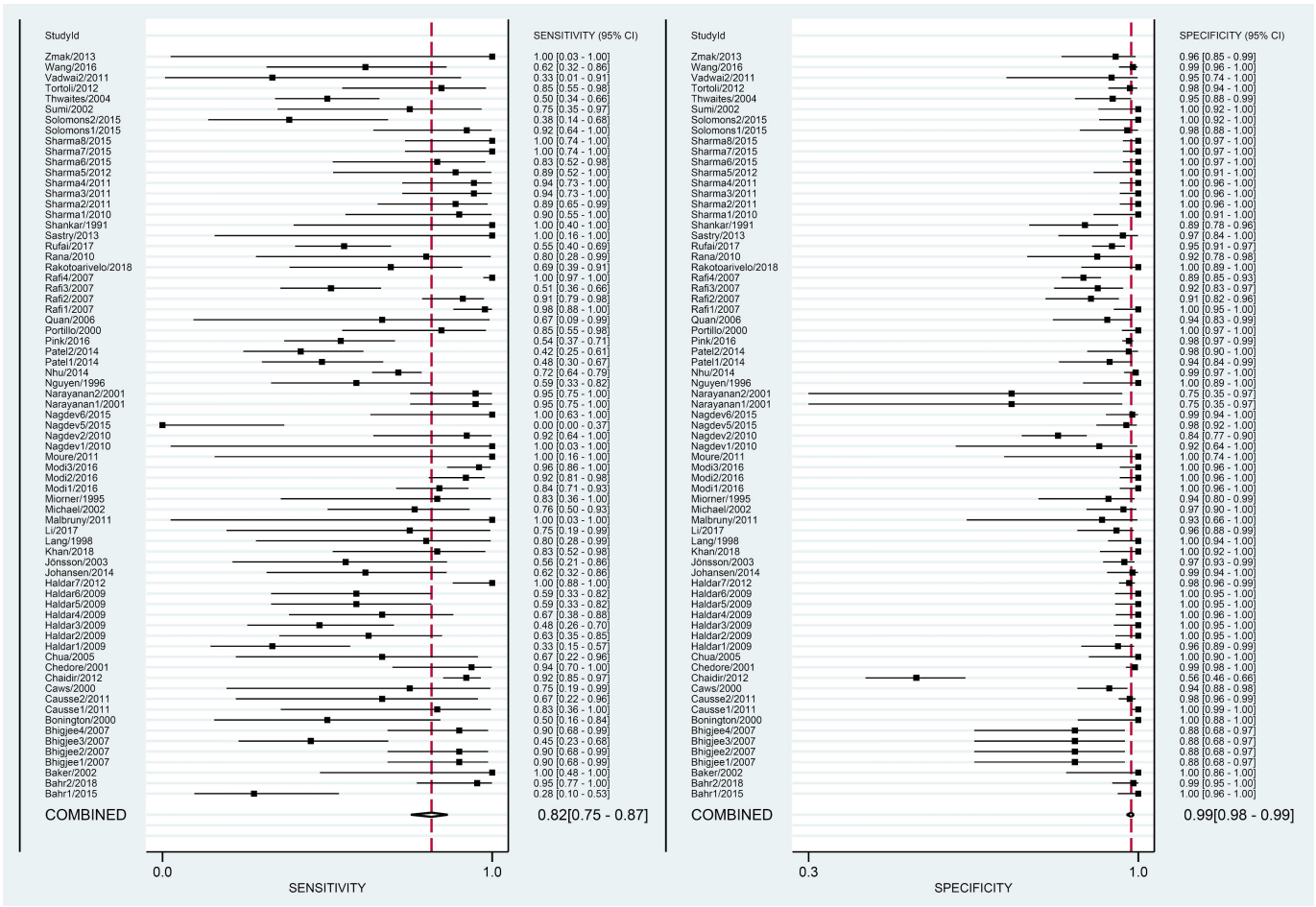

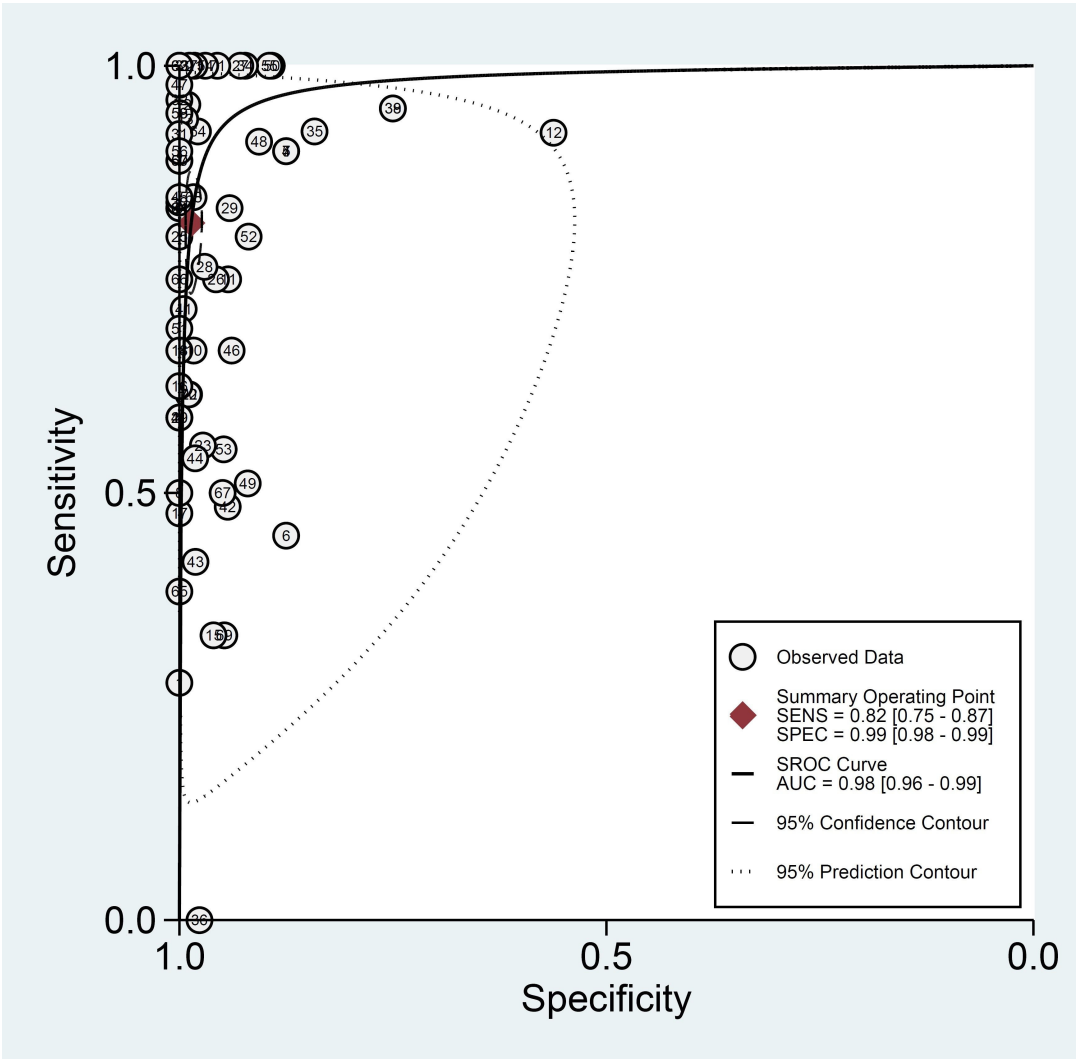

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