Improving early diagnosis of tuberculous meningitis in children

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4.1 Commercial nucleic acid amplification tests in tuberculous meningitis - a meta-analysis

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ABSTRACT

INTRODUCTION: Although nucleic acid amplification tests (NAATs) promise a rapid, definitive diagnosis of tuberculous meningitis, the performance of first-generation NAATs were sub-optimal and variable.

METHODS: We conducted a meta-analysis of studies published between 2003 and 2013, using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool to evaluate methodological quality. The diagnostic accuracy of newer commercial NAATs was assessed.

RESULTS: Pooled estimates of diagnostic accuracy for commercial NAATs measured against a cerebrospinal fluid *Mycobacterium tuberculosis* culture positive gold standard were; sensitivity 0.64, specificity 0.98 and diagostic odds ratio 64.0. Heterogeneity was limited; p value=0·147 and I^2 =33·85%. The Xpert MTB/RIF® test was evaluated in one retrospective study and four prospective studies, with pooled sensitivity 0.70 and specificity 0.97. The QUADAS-2 tool revealed low risk of bias, as well as low concerns regarding applicability. Heterogeneity was pronounced among studies of in-house tests.

CONCLUSIONS: Commercial NAATs proved to be highly specific with greatly reduced heterogeneity compared to in-house tests. Sub-optimal sensitivity remains a limitation.

Keywords: central nervous system; tuberculosis; nucleic acid amplification tests; diagnostic accuracy

INTRODUCTION

In 1993, the World Health Organization (WHO) declared tuberculosis (TB) a global public health emergency, with an estimated 7-8 million cases and 1.3-1.6 million TB deaths per year. By 2012, the situation has improved in many areas, but absolute numbers remain virtually unchanged with an estimated 8.7 million new cases and 1.4 million TB deaths.[1] Central nervous system (CNS) involvement, mostly tuberculous meningitis (TBM), accounted for approximately 1% of all TB cases.[2] In fact, TBM has been reported as the most common form of meningitis diagnosed in children from TB endemic areas with access to expanded program of vaccination (EPI) vaccines, including *Haemophilus influenza* type-B and pneumococcal vaccination.[3] Delayed diagnosis of TBM is universally associated with poor treatment outcome.[4]

The early clinical presentation of TBM is often non-specific with symptoms such as cough, loss of weight, fever, vomiting and malaise. As the disease progresses, more specific features such as meningism, focal neurological signs, convulsions and depressed level of consciousness occur.[5] TBM outcome is often poor despite adequate anti-mycobacterial therapy, due to irreversible damage preceding delayed diagnosis and ongoing immune-mediated pathology on treatment. Early treatment initiation is critical to reduce TBM-associated morbidity, mortality and healthcare costs, emphasizing the importance of early and accurate diagnosis.[6,7]

Culture of *Mycobacterium tuberculosis* (*M.tb*) from cerebrospinal fluid (CSF) is regarded as the most definitive diagnosis, although this is rarely attained. TBM is a paucibacillary disease. This could explain that direct microscopy for acid-fast bacilli in CSF is rarely positive,[8] while mycobacterial culture may take up to 42 days and has limited sensitivity (<50%) compared to clinical criteria.[5,9,10] In clinical practice the diagnosis of TBM is usually based on a combination of clinical, laboratory and radiological findings. The use of uniform case definition categories has been proposed for research purposes[11] with "definite TBM" defined as a positive CSF *M.tb* culture and/or commercial nucleic acid amplification test (NAAT).

NAATs have been introduced to provide rapid TB diagnosis and enhanced sensitivity compared to smear microscopy.[4,12-15] Although primarily developed for the analysis of respiratory specimens, these methods are often used in non-respiratory specimens as well.[13,14,16-18] They are presumed to be highly specific,[11,19] since they detect *M.tb*-specific DNA sequences such as the IS6110 insertion element, MBP64, 65 kDa antigen, and the rpoB region.[20,21]

In 2003 a systematic review evaluated the test accuracy of NAATs in the diagnosis of TBM.[18] The authors included 49 studies published between 1990 and 2002; both commercial and in-house NAATs were evaluated. The 14 studies with commercial NAATs revealed a pooled sensitivity and specificity of 56% and 98%, respectively. Summary accuracy measures of 35 studies with in-house NAATs could not be determined due to heterogeneity of the tests. Reasons for heterogeneity included: 1) inadequate standardization of laboratory techniques, 2) use of highly variable reference standards, 3) and small patient numbers with limited statistical power.[4] The review concluded that commercial NAATs provided valuable information when positive, but due to poor sensitivity a negative test did not exclude TBM.[18] This finding motivated the inclusion of a positive commercial NAAT as a marker of "definite TBM" in a proposed uniform TBM case definition for use in clinical research.[11]

Since then, many additional studies evaluated the use of commercial NAATs in the diagnosis of TBM, but no updated meta-analysis has been performed. We performed a systematic review of all recent studies (published since 2003) that evaluated the use of NAATs to diagnose TBM, with particular emphasis on commercial tests including the Xpert MTB/RIF® test.

METHODS

We identified all studies published between January 2003 and April 2013 from the following online databases: PubMed (MedLine), Web of Knowledge, Scopus and LILACS. Search terms used were: "Tuberculosis, Central Nervous System", "Tuberculoma, Intracranial", "Tuberculosis", "Mycobacterium tuberculosis", "Extrapulmonary tuberculosis", "Tuberculous meningitis", "Tuberculous pachymeningitis", "Central nervous system" and/or "Kochs disease" and "Polymerase Chain Reaction", "Ligase chain reaction", "GeneXpert" and/or " Nucleic acid amplification testing". Only articles written in English were included. Case reports and review articles were excluded. Studies with less than 10 subjects were also excluded. References of selected articles were reviewed to identify additional eligible studies. Three reviewers (RS, SLvE and AMvF) independently evaluated study inclusion; differences were resolved by consensus.

Data extraction

Two reviewers (RS and SLvE) independently extracted data including number of cases, number of controls, reference standard used, type of NAAT evaluated. Diagnostic odds ratios were extracted or calculated from the data provided. Differences

were resolved by consensus. Methodological quality was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool.[23-25]

Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences version 19 (SPSS Inc, Chicago, IL, USA), Comprehensive Meta Analysis version 2 (Biostat, Eaglewood, NJ, USA) and Meta-DiSc (Unit of Clinical Biostatistics, Ramón y Cajal Hospital, Madrid, Spain). Sensitivity, specificity and diagnostic odds ratio (DOR) were computed for each of the included studies. Pooled summary effect estimates were calculated, using a random effects model. Where both CSF culture and clinical criteria were analyzed separately as reference standards, only the studies with CSF culture as the reference standard were included. When articles evaluated more than one NAAT, or more than one quality measure, these were analyzed separately.

Receiver operating characteristic (ROC) curves based on either the regression of logit sensitivity on specificity, the regression of logit specificity on sensitivity, or an orthogonal regression line by minimizing the perpendicular distances were derived. These lines were transformed back to the original ROC scale to obtain a summary ROC (SROC) curve. Derived logit estimates of sensitivity, specificity and respective variances were used to construct a hierarchical SROC curve with these summary estimates. The area under the curve serves as a global measure of test performance; a value of 1 indicates perfect accuracy.[26] Heterogeneity was assessed by applying the X^2 homogeneity test to calculated odds ratios (as a single measure) and determining I^2, with values of more than 50% indicating heterogeneity.[26-28] Statistical significance was set at 0.05 for heterogeneity testing.

RESULTS

The study selection process is summarized in Figure 1. The literature search revealed 1125 potential articles, which was narrowed down to 69 articles after title screening. This was narrowed down further to 62 articles after abstract screening. Thirty-six articles were excluded after screening the text, and 4 articles added after cross referencing. Ten studies in 8 articles, describing commercial tests were selected; 40 studies in 22 articles describing in-house NAATs were tabulated seperately[4,8,14,15,19,21,28-48-53] (Supplementary table 1). Reference standards used in the ten studies evaluating commercial NAATs included a positive CSF *M.tb* culture in nine (90%) and clinical criteria in one (10%). To avoid misleading results, only the 9 commercial studies with positive CSF *M.tb* culture as the reference standard were analyzed. A variety of

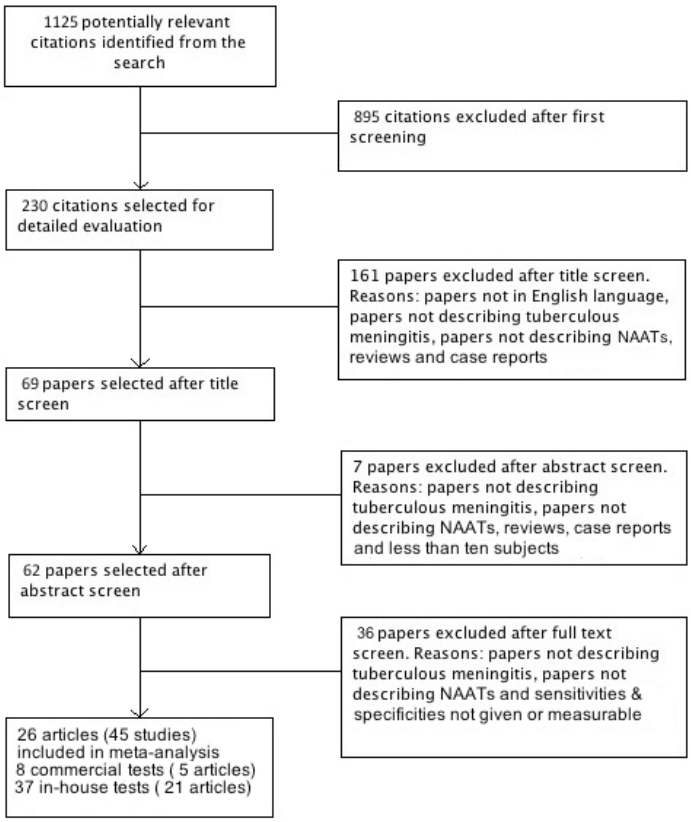

Figure 1. Flow diagram of all studies identified and those selected for meta-analysis

DNA extraction techniques and target sequences were used. Table 1 summarizes key characteristics of the commercial NAAT studies. Figure 2 reflects formal assessment of the four study domains evaluated by the QUADAS-2 tool; inter-reviewer variability using the tool was 10.6%.[24]

Summary test accuracy estimates for the nine commercial NAATS evaluated were; sensitivity 0.64 (95% CI 0.56-0.72), specificity 0.98 (95% CI 0.96-0.99), positive likelihood ratio 20.36 (95% CI 11.29-36.73), negative likelihood ratio 0.39 (95% CI 0.30-0.53) and DOR 64.0 (95% CI 26.9-152.1). Heterogeneity was limited; p value=0.147 and I^2 =33.85%. Table 2 shows heterogeneity testing after stratification of the commercial NAATs based on study design, prospective nature and Xpert MTB/RIF testing. Figure 3 provides an overview of sensitivities and specificities of commercial NAATs in forest plot format. Figure 4 presents the SROC curve for the commercial NAAT studies combined, with the respective studies presented as circles. The area under the curve (AUC) for all commercial tests combined was 0.92.

Table 1. Characteristics of commercial nucleic acid amplification test studies included in the meta-analysis

tAuthor	Study design	Reference standard	NAAT used	Sensitivity (95% CI)	Specificity (95% CI)
Johnsson[9]	Retrospective case-control	Clinical criteria	Cobas Amplicor	0·56(0·21-0·86)	0·97(0·93-0·99)
Johansen[29]	Prospective cross-sectional	CSF culture	standard BD ProbeTec ET	0·62(0·32-0·86)	0·99(0·94-1·00)
Johansen[29]	Prospective cross-sectional	CSF culture	modified BD ProbeTec ET	0·77(0·46-0·95)	0·99(0·94-1·00)
Thwaites[4]	Retrospective case-control	CSF culture	enhanced MTD	0·50(0·34-0·66)	0·95(0·88-0·99)
Causse[50]	Prospective cross-sectional	CSF culture	Xpert MTB/Rif	0·83(0·36-1·00)	1.00(0·92-1·00)
Causse[50]	Prospective cross-sectional	CSF culture	Cobas Taqman MTB	0·67(0·22-0·96)	0·98(0·88-1·00)
Malbruny[51]	Prospective cross-sectional	CSF culture	Xpert MTB/Rif	1·00(0·03-1·00)	1·00(0·77-1·00)
Vadwai[52]	Prospective cross-sectional	CSF culture	Xpert MTB/Rif	0·33(0·01-0·91)	0·95(0·74-1·00)
Tortoli[47]	Retrospective case-control	CSF culture	Xpert MTB/Rif	0.85(0·55-0·98)	0.98(0·94-1·00)
Patel[53]	Prospective cross-sectional	CSF culture	Xpert MTB/Rif	0·67(0·53-0·79)	0·94(0·85-0·98)

CI= confidence interval, CSF= cerebrospinal fluid

*NAAT used: Cobas amplicor (Roche Molecular Systems, Branchburg, NJ, USA), BD ProbeTec ET assay (Becton, Dickinson and Company, Sparks, MD, USA), MTD (Gen-Probe Inc, San Diego, Ca, USA), Genei Amplification kit (Bangalore Genei, Banglore, India), Xpert MTB/RIF (Xpert) (Cepheid, Sunnyvale, CA, USA)

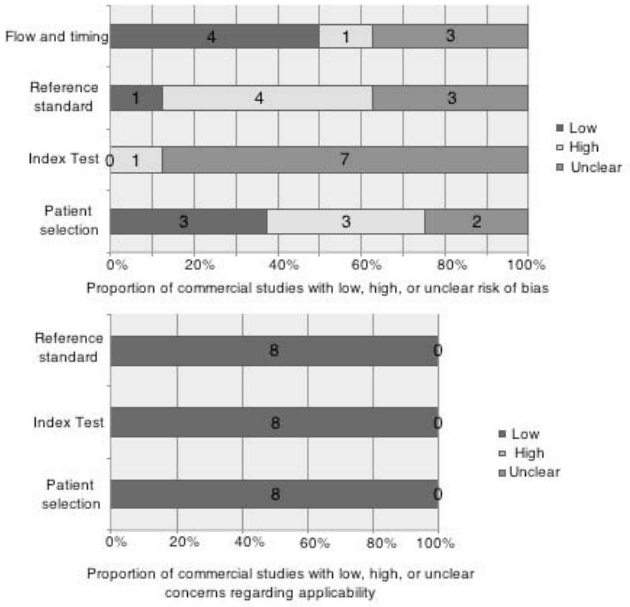

Figure 2. Bar graphs representing quality assessment by the QUADAS-2 tool[24]
The numbers in the bars represent the individual commercial NAATs

Table 2. Heterogeneity testing of commercial NAATs in stratified sub-groups

Subgroup	Number of studies	Summary DOR	95% CI	Test for heterogeneity p-value	I^2 (%)
Study design					
Case-control	2	68.5	4.3-1106.8	0.018	82.06
Cross-sectional	7	59.8	26.2-136.2	0.416	1.08
Prospective data collection					
Yes	7	59.8	26.2-136.2	0.416	1.08
No	2	68.5	4.3-1106.8	0.018	82.06
PCR type					
Xpert MTB/Rif	5	70.7	17.4-287.1	0.157	39.65

DOR= diagnostic odds ratio, CI= confidence interval, I^2 is a measure of heterogeneity (>50%= heterogeneity)

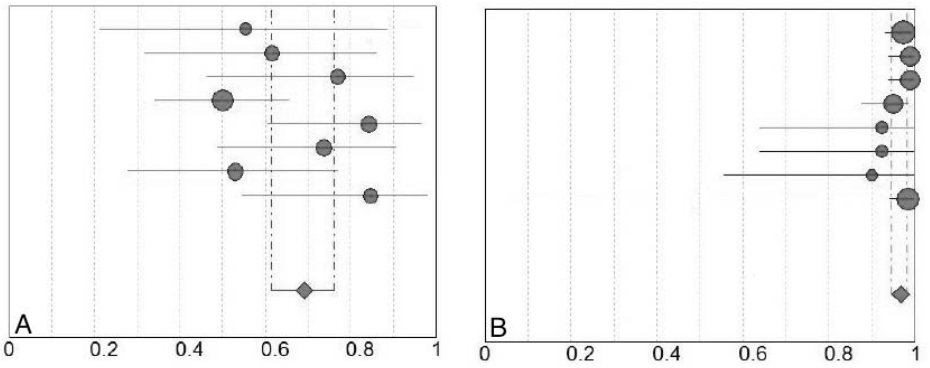

Figure 3. Forest plots of A) sensitivity and B) specificity of commercial NAATs
Each circle shows the point estimate of sensitivity and specificity from each included study. Error bars represent 95% confidence intervals.

Summary test accuracy estimates for the 40 in-house tests revealed sensitivity of 0.73 (95% CI 0.71-0.75), specificity of 0.92 (95% CI 0.90-0.93), positive likelihood ratio of 9.56 (95% CI 6.61-13.84), negative likelihood ratio of 0.27 (95% CI 0.20-0.35) and DOR of 40.6 (95% CI 26.6-61.9). Heterogeneity was pronounced; p-value=0.001 and I^2 = 58·86%. Supplementary table 2 shows heterogeneity testing after stratification of the in-house NAATs based on study design, prospective nature, randomization, blinding, reference standard and type of PCR used. Forest plots of sensitivities and specificities of in-house NAATs and the relevant SROC plot are included in Supplementary figure 1. The AUC for all in-house tests combined was 0.94.

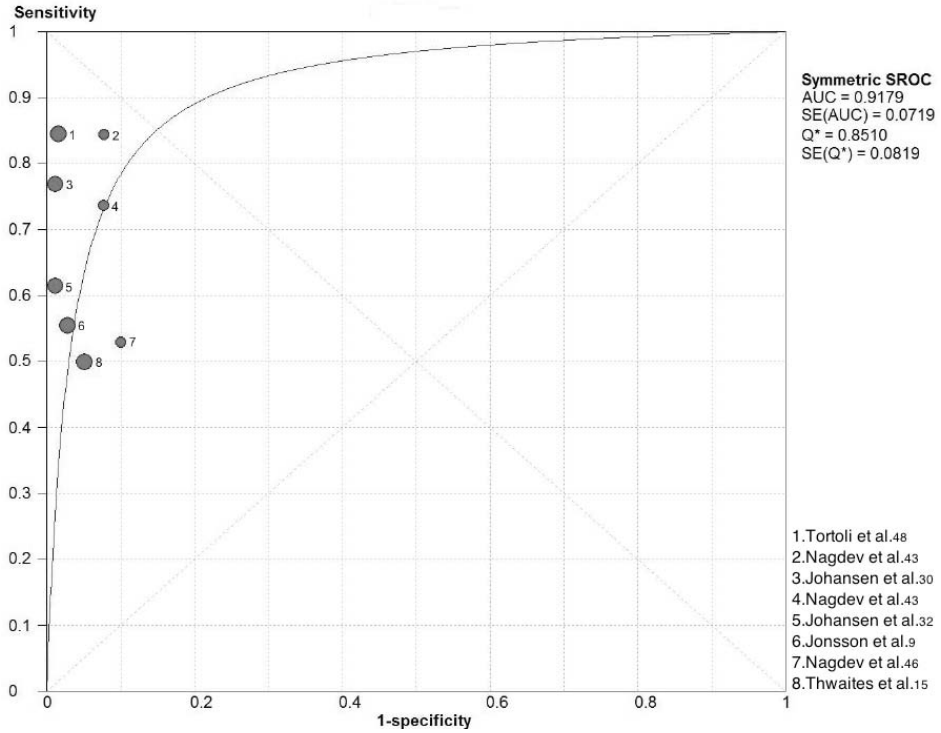

Figure 4. SROC curve for commercial NAATs
Each study is represented by a circle (the size of the circle reflecting study size) and the dark line shows the summary diagnostic accuracy.

DISCUSSION

The need for a test that can diagnose TBM rapidly and accurately, especially during its early phases, is self-evident.[54] Our systematic review and meta-analysis of commercial NAATs with a CSF *M.tb* culture-positive gold standard found a summary sensitivity estimate of 0.64. Unfortunately this remains suboptimal and is unlikely to greatly enhance early accurate diagnosis, since most study specimens were collected from patients with advanced TBM disease. In contrast, commercial NAATs exhibited excellent specificity of 0·98 and in the correct clinical context it may be regarded as a definitive test.[11] Similar to findings from the previous systematic review performed in 2003,[18] we found significant heterogeneity among in-house NAATs and consistent performance in the commercial group. When comparing our summary estimates to the previous meta-analysis, the sensitivity of more recent commercial NAATs shows improvement (0.64 vs 0.56) with a similar specificity (0.98). Our summary estimate of negative likelihood ratio is lower (0.39 vs 0.44), but still far from ideal when considering its use as a "rule-out" test.

Despite suboptimal sensitivity, the rapid turnaround time of NAATs compared to culture enhances its role in the early accurate diagnosis of TBM. However, most commercial NAATs are validated for pulmonary samples and are still not advised for routine diagnostic use.[47] The Xpert MTB/RIF assay (Cepheid, CA, USA) has been endorsed by the WHO for use on both smear positive and negative respiratory specimens. The findings of the Xpert MTB/RIF assay demonstrated rapid diagnosis in a large retrospective study of extrapulmonary specimens, including an encouraging sensitivity of 0.85 and specificity 0.98 for CSF samples.[47] When combined with the 4 prospective studies testing the Xpert MTB/RIF assay in CSF samples, a pooled sensitivity and specificity of 0.70 and 0.97 was obtained.[47,50-53] Provided that similar measures of sensitivity and specificity can be maintained in future studies using the Xpert MTB/RIF assay in CSF specimens, the goal of Xpert MTB/RIF assay as a "stand alone" test for diagnosis of TBM can be achieved.

The use of microscopy, culture and NAATs together with clinical features and neuroimaging in a pragmatic algorithm seems preferable to improve diagnostic accuracy. This updated meta-analysis supports the conclusion that a positive commercial NAAT result provides a definite TBM diagnosis in the right clinical context, as suggested in the proposed uniform research case definition for TBM in adults and children.[11] The rest of the components of the proposed uniform research case definition can compensate for commercial NAATs when excluding a diagnosis of TBM.

The 'gold standard' for the diagnosis of TBM is the identification of *M.tb* on CSF culture or identification of acid-fast bacilli on CSF microscopy. The low sensitivity of both these methods has prompted leading researchers to use alternate clinical reference standards. In our meta-analysis we attempted to avoid overestimating summary estimates of diagnostic accuracy by only analyzing commercial NAATs using an *M.tb* culture positive reference standard. The low heterogeneity observed when studies were prospective or cross-sectional was also encouraging (Table 2). Similar to previous findings, in-house NAAT studies demonstrated excessive heterogeneity with wide variability in methodological quality (Supplementary table 2).[18]

The quality and reporting of diagnostic accuracy studies on commercial tests for TB, malaria and HIV are problematic.[25] To minimize these concerns, screening and selection of articles were assessed by three independent reviewers followed by rigorous quality assessment using the QUADAS-2 tool.[24] This resulted in multiple study exclusions, but careful assessment of study accuracy and reliability strengthens the findings of our meta-analysis. Overall, the studies revealed a low risk of bias in the

categories of flow and timing, reference standard, index test and patient selection. There was little concern regarding the applicability of study findings.

In conclusion, commercial NAATs revealed good specificity and postive predictive values for the diagnosis of TBM on CSF samples in areas of high TB prevalence. However, sensitivity and negative predictive values remain suboptimal, hampering the ability to direct treatment, especially early in the disease process when the best treatment outcomes can be achieved.

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CONFLICT OF INTERESTS

None of the authors have any conflict of interests.

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Supplementary table 1. Characteristics of in-house studies included in the meta-analysis

Author	Study design	Reference standard	NAAT used	Sensitivity (95% CI)	Specificity (95% CI)
Rafi[15]	Retrospective case-control study	Clinical criteria	PCR 123 bp	0·86(0·68-0·96)	1·00(0·54-1·00)
Kulkarni[30]	Retrospective case-control study	Clinical criteria	PCR 340 bp So hybridization	0·90(0·73-0·98)	1·00(0·88-1·00)
Kulkarni[30]	Retrospective case-control study	Clinical criteria	PCR 340 bp eth. bromide	0·73(0·54-0·88)	1·00(0·88-1·00)
Desai[31]	Retrospective case-control study	Clinical criteria	PCR IS*6110* QIAmp	0·67(0·47-0·83)	1·00(0·87-1·00)
Desai[31]	Retrospective case-control study	Clinical criteria	PCR IS*6110* CTAB	0·50(0·31-0·69)	1·00(0·87-1·00)
Juan[32]	Retrospective case-control study	CSF micro/culture /clinical criteria	PCR IS*6110*	0·68(0·41-0·85)	0·99(0·94-1·00)
Quan[33]	Retrospective case-control study	Clinical criteria	PCR IS*6110*	0·75(0·53-0·90)	0·94(0·83-0·99)
Bhigjee[34]	Retrospective cross-sect study	CSF culture/ clinical criteria	PCR IS*6110*, MBP64, PT8/9	0·55(0·39-0·70)	0·88(0·68-0·97)
Bhigjee[34]	Retrospective cross-sect study	CSF culture/ clinical criteria	PCR (real-time) IS*6110*	0·70(0·55-0·83)	0·88(0·68-0·97)
Deshpande[35]	Retrospective case-control study	CSF culture	PCR IS*6110*	0·91(0·77-0·98)	0·76(0·56-0·90)
Rafi[36]	Retrospective case-control study	CSF culture	PCR IS*6110*	0·98(0·88-1·00)	1·00(0·95-1·00)
Rafi[36]	Retrospective case-control study	CSF culture	PCR MBP64	0·91(0·79-0·98)	0·91(0·82-0·96)
Rafi[36]	Retrospective case-control study	CSF culture	PCR 65 kDa	0·51(0·36-0·66)	0·92(0·83-0·97)
Rafi[20]	Prospective cohort study	CSF culture	PCR IS*6110*	1·00(0·97-1·00)	0·89(0·85-0·93)
Dora[37]	Prospective cross-sectional study	CSF culture	in-house PCR 65kDA nested	0·50(0·12-0·88)	0·99(0·95-1·00)
Takahashi[38]	Prospective cross-sectional study	Clinical criteria	PCR single MBP64	0·40(0·05-0·85)	1·00(0·48-1·00)
Takahashi[38]	Prospective cross-sectional study	Clinical criteria	PCR nested MBP64	1·00(0·48-1·00)	1·00(0·48-1·00)
Takahashi[38]	Prospective cross-sectional study	Clinical criteria	PCR OR-QNRT	1·00(0·48-1·00)	1·00(0·48-1·00)
Takahashi[38]	Prospective cross-sectional study	Clinical criteria	PCR WR-QNRT	1·00(0·48-1·00)	1·00(0·48-1·00)
Haldar[39]	Retrospective case-control study	CSF culture/ clinical criteria	sediment PCR qRT	0·53(0·42-0·64)	0·92(0·84-0·97)
Haldar[39]	Retrospective case-control study	CSF culture/ clinical criteria	sediment PCR devR	0·31(0·21-0·42)	0·94(0·87-0·98)

Author	Study design	Reference standard	NAAT used	Sensitivity (95% CI)	Specificity (95% CI)
Haldar[39]	Retrospective case-control study	CSF culture/ clinical criteria	sediment PCR IS*6110*	0·40(0·29-0·51)	0·93(0·85-0·97)
Haldar[39]	Retrospective case-control study	CSF culture/ clinical criteria	filtrate PCR qRT	0·88(0·78-0·94)	0·92(0·84-0·97)
Haldar[39]	Retrospective case-control study	CSF culture/ clinical criteria	filtrate PCR devR	0·88(0·78-0·94)	0·87(0·78-0·93)
Haldar[39]	Retrospective case-control study	CSF culture/ clinical criteria	filtrate PCR IS*6110*	0·85(0·76-0·92)	0·84(0·74-0·91)
Huang[40]	Retrospective case-control study	Clinical criteria	single PCR rpoB	0·25(0·12-0·42)	1·00(0·86-1·00)
Huang[40]	Retrospective case-control study	Clinical criteria	nested PCR rpoB	0·86(0·71-0·95)	1·00(0·86-1·00)
Rana[41]	Retrospective case-control study	CSF micro/ culture /clinical criteria	PCR IS*6110*	0·31(0·20-0·46)	0·92(0·78-0·98)
Nagdev[42]	Retrospective case-control	CSF culture or microscopy & clinical criteria	nested PCR IS*6110*- Genei Amplification kit Chelex protocol	0·84(0·60-0·97)	0·92(0·64-1·00)
Nagdev[42]	Retrospective case-control	CSF culture or microscopy & clinical criteria	nested PCR IS*6110*- Genei Amplification kit Phenol/ chloroform	0·74(0·49-0·91)	0·92(0·64-1·00)
Nagdev[43]	Retrospective case-control study	Sputum micro/ CSF culture/ clinical criteria	PCR IS*6110*	0·80(0·66-0·90)	0·84(0·77-0·90)
Sharma[44]	Retrospective case-control study	CSF culture/ clinical criteria	PCR protein b	0·83(0·72-0·91)	1·00(0·91-1·00)
Nagdev[45]	Retrospective case-control study	CSF micro/ culture /clinical criteria	PCR IS*6110*	0·88(0·64-0·99)	0·80(0·44-0·97)
Nagdev[45]	Retrospective case-control	Clinical criteria	nested PCR IS*6110*- Genei Amplification kit	0·53(0·28-0·77)	0·90(0·55-1·00)
Kusum[22]	Retrospective case-control study	CSF micro/ culture /clinical criteria	PCR IS*6110*	0·76(0·67-0·84)	1·00(0·96-1·00)
Kusum[22]	Retrospective case-control study	CSF micro/ culture /clinical criteria	PCR protein b	0·81(0·72-0·88)	1·00(0·96-1·00)
Kusum[22]	Retrospective case-control study	CSF micro/ culture /clinical criteria	PCR MBP64	0·83(0·74-0·89)	1·00(0·96-1·00)
Iacob[46]	Prospective cohort study	CSF culture/ clinical criteria	PCR IS*6110*	0.87(0·81-0·93)	0.88(0·82-0·94)

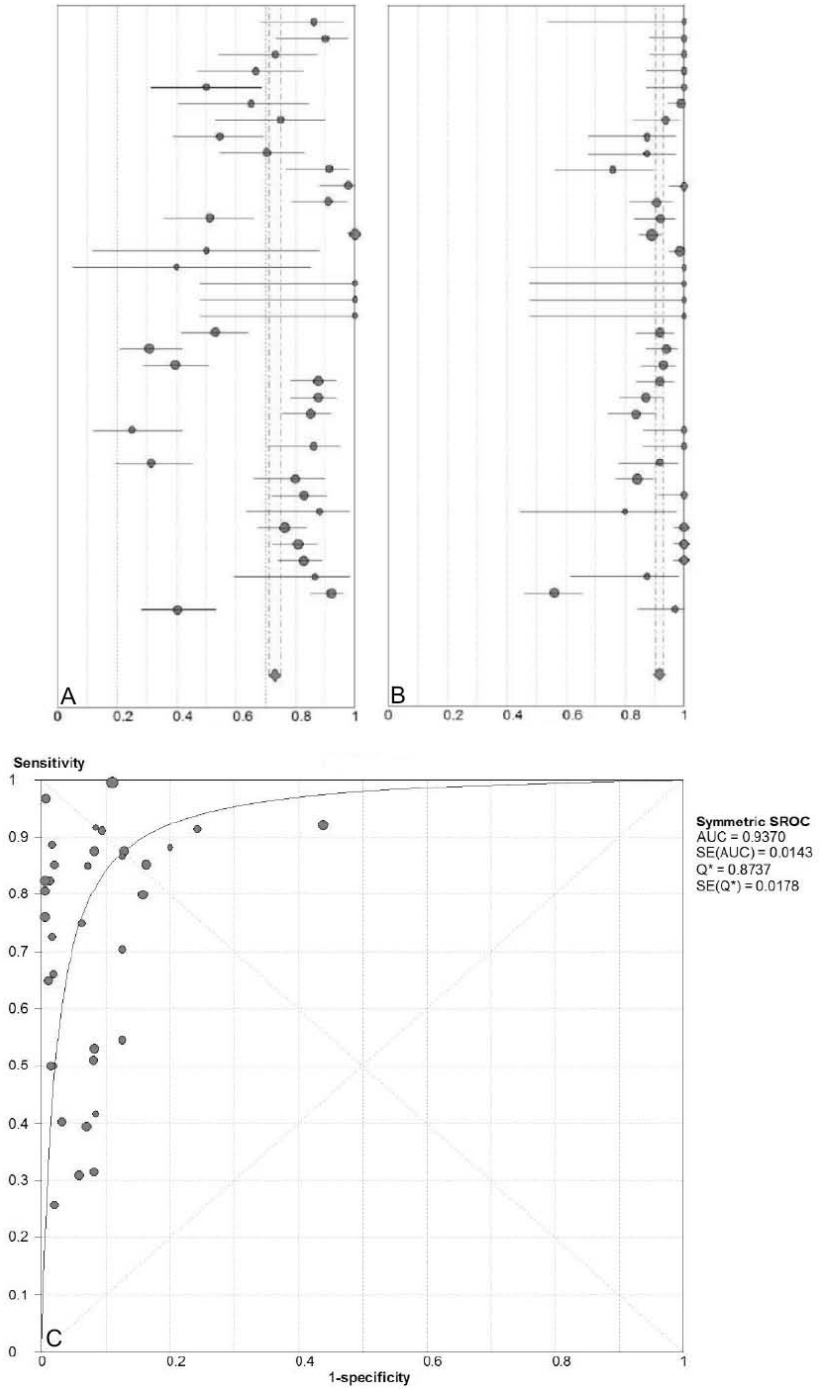
Author	Study design	Reference standard	NAAT used	Sensitivity (95% CI)	Specificity (95% CI)
Chaidir[49]	Prospective cohort study	CSF culture	PCR IS*6110*	0.92(0·90-0·94)	0.56(0·52-0·59)
Sastry[48]	Prospective cohort study	CSF culture/ clinical criteria	nested PCR IS*6110*	0.43(0·38-0·49)	0.97(0·95-0·99)

CI= confidence interval, CSF= cerebrospinal fluid, OR-QNRT= original quantitative nested real-time, WR-QNRT= wide-range quantitative nested real-time.

Supplementary table 2. Heterogeneity testing of in-house NAATs in stratified sub-groups

Subgroup	Number of studies	Summary DOR	95% CI	Test for heterogeneity p value	I^2(%)
Study design					
Case-control	29	44.7	27.1-73.8	0.000	63.43
Cross-sectional	7	19.3	8.7-42.8	0.479	0.00
Prospective data collection					
Yes	9	49.7	17.0-145.4	0.068	45.05
No	31	39.7	24.7-63.7	0.000	62.60
Randomization					
No	36	40.6	26.2-63.0	0.000	62.25
Blinding					
Single-blinded	10	106.4	27.1-417.6	0.002	66.34
Non-blinded	28	30.2	19.8-46.2	0.000	54.15
Reference standard					
Culture	7	72.2	21.3-245.5	0.000	78.65
Clinical criteria	13	57.7	26.9-123.5	0.743	0.00
CSF microscopy and/or culture and clinical criteria	20	31.0	18.1-53.2	0.000	64.39
PCR type					
IS*6110*	19	36.5	20.3-65.7	0.001	58.89
Other	21	46.2	24.6-86.7	0.000	60.19
Presence of nesting	7	42.0	16.8-104.8	0.648	0.00

DOR= Diagnostic odds ratio, CI= confidence interval, I^2 is a measure of heterogeneity (>50%= heterogeneity)



Supplementary Figure 1. Forest plots of A) sensitivity B) specificity and c) SROC curve of in-house NAATs

Each circle shows the point estimate of sensitivity and specificity from each included study.